Programming effects of adversity on adolescent adaptive capacity

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1

General introduction

Adverse life events are a well-known risk factor for an individual's vulnerability to the development of mental disorders later in life (Rutter, 2006). Extensive research has provided evidence that from early adolescence onwards, incident rates steeply rise for many common mental disorders (Bernstein, Borchardt, & Perwien, 1996; Hankin et al., 1998). These increases in combination with the major biological, psychological and social changes, typical for the adolescent years, raise the question if adolescence can be seen as a vulnerable period for the influence of adversity. Over the years scientists have become increasingly interested in the mechanisms by which adversity affects mental health. An important underlying mechanism could be adversity driven changes in adaptive capacity. Adaptive capacity reflects constitutional differences between people and can be used as an indicator of an individual's ability to adapt to the (changing) environment. This PhD thesis aims to study programming effects of adversity on changes in adaptive capacity. More specific, we explore whether and how exposure to adverse events can alter temperament and HPA-axis functioning during adolescence. In addition, differences in sensitivity to adversity and consequences of changes in adaptive capacity will be discussed.

Temperament and HPA-axis functioning in the transduction from adversity to psychopathology

Adaptive capacity can be studied at various levels. First, an extensively studied, psychological level of adaptive capacity is temperament, or personality. Temperament involves relatively stable individual differences in emotional, attentional and behavioural processes that emerge early in development (e.g., Rothbart, Ahadi, & Evans, 2000). Traditionally, temperament has been conceptualised as a predominantly biologically based precursor of later personality. Over the last years, an increasing emphasis on the connection between temperament and personality has emerged, suggesting that they are largely equivalent and the terms may be used interchangeably (Caspi & Shiner, 2006; Costa & McCrae, 2001; Klein, Kotov, & Buffered, 2011). Therefore, although we use both terms throughout this thesis (depending on the study, questionnaire or reference), we will be referring to the same construct. Numerous studies have provided evidence for the association between temperament and psychopathology (e.g., Kotov, Gamez, Schmidt, & Watson, 2010). Moreover, temperament has been suggested to increase risk, or provide protection for the influence of stressful events on the development of mental problems.

Second, adaptive capacity can also be studied at a physiological level, such as cardiovascular or hypothalamic-pituitary-adrenal (HPA) axis functioning. The current thesis focuses on HPA-axis functioning. The HPA-axis is one of the key components in the body's stress system. Activation of the HPA-axis results in a release of cortisol from the adrenal cortex. HPA-axis functioning can be studied in terms of basal levels, increases after waking up in the morning, or responses to acute stress. Like temperament, cortisol has been implicated in the transduction of adversity into psychopathology (e.g., Burke, Davis, Otte, & Mohr, 2005).

Findings on adversity, HPA-axis functioning and psychopathology are, however, inconsistent. As a result, little is known about the nature of these associations.

Stability and change in temperament and HPA-axis functioning

The notion of adaptive capacity (both in terms of temperament and HPA-axis functioning), as characterizing constitutional differences between people, implies a certain level of stability. Consequently, both temperament and HPA-axis functioning have been studied as predictor rather than outcome variables. Indeed, behavioural genetic studies have provided evidence for a substantial 'stability' component in temperament (often operationalized as broader personality traits, Kandler et al., 2010). Nonetheless, extensive literature has also demonstrated that traits are not developmentally stable (McCrae et al., 2002; Roberts, Wood, & Smith, 2005; Robins, Noftle, Trzesniewski, & Roberts, 2005).

Less is known about stability and change in HPA-axis functioning. Only a single study has investigated functioning of the HPA-axis using a longitudinal design, suggesting small increases in basal cortisol from childhood to late adolescence (Trickett, Noll, Susman, Shenk, & Putnam, 2010). So far, stress-induced cortisol has not been assessed longitudinally. Nonetheless, from twin studies we know that the genetic component is, similar to heritability of temperament, substantial for basal cortisol, but only modest for stress-induced cortisol levels (Bartels, Van den Berg, Sluyter, Boomsma, & de Geus, 2003; Riese, Rijsdijk, Rosmalen, Snieder, & Ormel, 2009; Wüst, Federenko, Hellhammer, & Kirschbaum, 2000). Moreover, support has been provided for a distinction between aspects of HPA-axis functioning in terms of trait and state components. Basal cortisol has been suggested to be a relatively stable, trait-like characteristic (Hellhammer et al., 2007), whereas stress induced cortisol is largely situation dependent, and may therefore be seen as a mainly state-like characteristic. This may suggest that the potential to change might be stronger for stress-induced cortisol, than for basal levels of HPA-axis functioning, but so far this has not been tested directly.

Adversity and changes in temperament and HPA-axis functioning

Although evidence has been provided for changes in temperament and (basal) HPA-axis functioning, research into *environmental influences* on these changes is limited. A few studies have examined sources of temperament change, either in terms of intrinsic maturational factors (Roberts et al., 2005), or in terms of environmental factors, such as stressful life events (Löckenhoff, Terracciano, Patriciu, Eaton, & Costa, 2009; Vaidya, Gray, Haig, & Watson, 2002). However, these studies tended to focus on adult samples and subsequently, the findings may or may not generalize to adolescents.

Even less is known about sources of change in HPA-axis functioning. Some cross-sectional studies have demonstrated associations between adversity and HPA-axis functioning (both during adolescence and adulthood; Heim & Nemeroff, 2001; Kaufman et al., 2000; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Sanchez, 2006). Additionally, a few retrospective studies

have assessed adult HPA-axis functioning and early life adversity. Findings from these studies, however, tend to be inconsistent (Bruce, Fisher, Pears, & Levine, 2009; Cicchetti & Rogosch, 2001; Elzinga et al., 2008; Heim, Ehlert, & Hellhammer, 2000; Miller, Chen, & Zhou, 2007; Ouellet-Morin et al., 2011; Tyrka et al., 2008) and only a single longitudinal study seem to have been performed (Trickett et al., 2010). Consequently, it is unknown whether adversity has the potential to cause (long lasting) alterations in HPA-axis functioning during adolescence. Some animal and human research has provided evidence that prenatal adversity can program infant's neurobiology (including HPA-axis functioning, Glover, O'Connor, & O'Donnell, 2010). The current thesis aims to investigate whether these findings on prenatal adversity generalize to adolescence; that is, to test longitudinally whether adversity during adolescence is related to changes in HPA-axis functioning.

In addition to the issues mentioned above, the largest gaps in our knowledge seem to pertain to the exact nature of the associations between adversity and changes in both temperament and HPA-axis functioning. Little is known, for example, on which aspects of adversity are related to temperament and HPA-axis functioning. With regard to adversity and temperament, studies have been limited to research on either severe traumatic events (e.g., loss of significant others; Mroczek & Spiro, 2003) or adult work or marriage related problems (e.g., Roberts, Caspi, & Moffitt, 2003). Consequently, it is unknown whether different types of events are differentially associated with temperament change, or whether multiple events can accumulate and therefore interfere stronger with development than exposure to a single event. With regard to HPA-axis functioning, sightly more seems to be known about which adversity characteristics are related to inter-individual variability in functioning based on cross-sectional and retrospective studies. In particular, events that threaten integrity (e.g., abuse, maltreatment), and that are uncontrollable, unpredictable, and chronic may be related to defiant HPA-axis functioning (Miller et al., 2007).

Given that both temperament and HPA-axis functioning are multi-dimensional concepts, - that is, different temperament traits and facets of HPA-axis functioning can be distinguished -, the question rises whether all aspects of temperament and HPA-axis functioning are equally sensitive to adverse events. Because of all traits neuroticism has been shown to correlate most strongly with psychopathology (e.g., Kotov et al., 2010); it might be that this trait is also more likely to show adversity-driven changes. Research on adversity and personality has indeed pointed into this direction by focusing on traits related to neuroticism (emotional instability, anxiety etc), but studies directly comparing the effects of stress on different traits are rare (Löckenhoff et al., 2009). Also with regard to HPA-axis functioning, no studies seem to have directly compared different aspects. Because, as mentioned before, the heritable component of HPA-axis functioning is substantially lower for stress-induced activity than for basal levels and because stress-induced cortisol has been suggested to be a more state-like characteristic than basal cortisol, it seems reasonable to expect that stress-induced activity is more likely to be affected by adversity. In addition, almost all studies on stress-induced

cortisol have focussed on increases in cortisol induced by a social stress task (e.g., a public speaking task), that is, stress-induced HPA-axis reactivity. However, physiologically, HPA-axis reactivity is primarily a marker for energy mobilization, and subsequently, effort rather than stress related (Koolhaas et al., 2011). Consequently, HPA-axis reactivity might not be the most interesting measure of stress-induced HPA-axis functioning in the context of adversity (and psychopathology). In contrast to reactivity, anticipatory activation, reflecting environmental unpredictability, and/or recovery after a stress-task, indicating lack of control over the situation, might be better indicators of (mal)adaptive functioning of the HPA-axis (Koolhaas et al., 2011). Since unpredictability and uncontrollability are the main determinants of stressful situations (Koolhaas et al., 2011), it might be more valuable to investigate the association between adolescent adversity and changes in anticipation to and/or recovery after a stress task, than between adolescent adversity and the traditionally used stress-induced HPA-axis reactivity.

Differential sensitivity to adversity

Over the last decade, an increasing emphasis has emerged on inter-individual differences in sensitivity to adversity. Several theoretical frameworks have proposed mechanisms that may account for this differential sensitivity. For example, risk exposures may accumulate and amplify the impact of (subsequent) stress on 'sensitive individuals' (the 'Diathesis Stress Model (Monroe & Simons, 1991). Recent research has suggested that, in addition to suffering more from an adverse environment, sensitive or susceptible children may also benefit relatively more from a supportive environment (the 'Differential Susceptibility' and 'Biological Sensitivity to Context' Models; Belsky & Pluess, 2009; Boyce & Ellis, 2005; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van Ijzendoorn, 2011). These models imply that individual characteristics can moderate the association between environmental influences and child outcomes, making certain children more sensitive than others, probably for better and certainly for worse. Nonetheless, debate remains about the factors that might moderate the link between stress and well-being and the robustness of this effect.

Given that temperament and HPA-axis functioning are generally studied as sensitivity markers themselves (e.g., in the associations between adversity and psychopathology), and not as outcome variables, little is known on whether individuals also differ with respect to the impact of adversity on temperament and HPA-axis functioning during adolescence. In addition to the well-established moderators in the adversity-psychopathology association (Ormel & Wohlfarth, 1991; Kendler et al., 2001; Rutter, 2000), some candidates for differential sensitivity to adversity have also been suggested with regard to more constitutional characteristics. For example, the interaction between early life adversity and genotype appears to create stable individual differences in neurobiology (e.g., the HPA-axis, Ladd, Huot, Thrivikraman, Nemeroff, & Plotsky, 2004). Little is known on whether similar variables also moderate the association between adolescent adversity and HPA-axis functioning or temperament. These might include genotype, but also other individual characteristics (e.g., gender) and environmental factors (e.g., pre- and postnatal adversity; social support).

Consequences of changes in adaptive capacity

Finally, when studying whether adversity predicts changes in relatively fundamental characteristics like temperament and HPA-axis functioning, the question rises how these changes are related to future well-being. Some evidence suggests that adversity might not only predict subsequent temperament, but that temperament can also predict future stress exposure. In addition, it might be that changes in adaptive capacity, either in terms of temperament or in terms of HPA-axis functioning, are related to the development of psychopathology. Of course, numerous studies have demonstrated the association between a single temperament measure and (future) psychopathology. In addition, particularly over the last decade, evidence has been mounting for an association between HPA-axis functioning and psychopathology (Adam et al., 2010; Goodyer, Bacon, Ban, Croudace, & Herbert, 2009; Sondeijker et al., 2008). It is unknown, however, about whether inter-individual variation in change (either temperament or HPA-axis functioning) predicts the development of mental disorders, above and beyond basal characteristics.

Project design

Data were used from the TRacking Adolescent's Individual Lives Survey (TRAILS), a prospective cohort study of Dutch adolescents who are followed biennially until they are at least 25 years old. The TRAILS target sample consisted of all preadolescents who lived in the northern part of the Netherlands during their recruitment at age 10, including both urban and rural areas. A detailed description of the study design, sampling procedures, data collection, and measures of the TRAILS study can be found in De Winter and colleagues (de Winter et al., 2005) and Huisman (Huisman, 2000). Temperament and personality were assessed with questionnaires at 3 waves. Data on HPA-axis functioning were collected by means of saliva, in the morning, to measure basal cortisol and cortisol awakening response and during a social stress task that was part of behavioural experiments to measure stress-induced cortisol responses (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). Novel to the literature so far, two waves of data on HPA-axis functioning were collected, when adolescents were 16 and again when they were 19 years old. Adversity was captured using elaborate interviews and questionnaires. Adverse events between age 11 and 16 were measured by means of the Event History Calendar (Caspi et al., 1996). Events between age 16 and 19 were measured by means of the Life History Interview (Kendler, Karkowski, & Prescott, 1998). In one study we also included childhood events, which were measured by means of a questionnaire that was filled in by the parent. Additionally, for Chapter 2 we used data from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPUD). Data for Chapter 7 came from the Avon Longitudinal Study of Parents and Children (ALSPAC).

Outline

The main aim of this thesis is to study programming effects of adversity on changes in temperament and HPA-axis functioning during adolescence (see Figure 1).

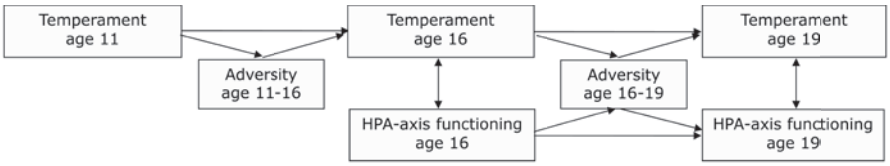

Figure 1. Schematic representation the main associations between adversity, temperament and HPA-axis functioning.

In Chapter 2 of this thesis, *"Genetic and environmental influences on the longitudinal structure of neuroticism: a Trait-State approach",* we will start with elucidating the longitudinal structure of the personality domain of neuroticism and quantify the genetic and environmental influences that contribute to stability and change using a behavioural genetic twin design and data from the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPUD). In Chapter 3, "*Adolescent personality: associations with basal, awakening and stress-induced cortisol responses",* cross-sectional associations between the main outcome domains of this thesis are investigated. Chapter 4 "*Stressful life-events and temperament change during early and middle adolescence"* and Chapter 5*"Normative and adversity-driven changes in hypothalamic-pituitary-adrenal axis functioning",* aim to examine the key research topic: programming effects of adversity on temperament and HPA-axis functioning. In Chapter 6, "*Stress-sensitivity and reciprocal associations between stressful events and temperament during adolescence",* we investigated bidirectional associations between adversity and temperament. In addition, given that large individual differences exist in sensitivity to environmental influences, various moderators of stress-sensitivity are explored including both moderators that have been well established in the adversity-psychopathology association and novel moderators such as prenatal adversity and a cumulative plasticity gene index. Chapter 7, "*Stressful events and psychological difficulties: testing alternative candidates for sensitivity",* continues about bidirectional associations and differential sensitivity, but by using data from the Avon Longitudinal Study of Parents and Children (ALSPAC), we move away from the relatively constitutional level of temperament and focus on the association between adversity and psychological difficulties. In the final chapter of this thesis (Chapter 8), *"A test of the Vulnerability Model: Temperament and temperament change as predictors of future mental disorders",* we aim to test whether changes in temperament are predictive of mental disorders a few years later, above and beyond basal temperament. Finally, in Chapter 9, we summarise the findings and integrate them to provide a new perspective on stability and change in temperament and HPA-axis functioning.

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Genetic and environmental influences on the longitudinal structure of neuroticism: a Trait-State approach

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ABSTRACT

Previous research has suggested both stability and change in personality. This study seeks to elucidate the longitudinal structure of neuroticism, using a behavioural genetic twin design. We tested whether this structure is best accounted for by a Trait-State, a Trait-only or a State-only model. In line with classic views on personality, a substantial Trait component was found; in addition, a substantial State component was detected. The contributions of genetic and environmental influences on the Trait component were nearly equal, whereas environmental influences on the State component were much stronger than genetic influences. Although the overall findings were similar for older and for younger twins, genetic influences on the Trait component were stronger than environmental influences in younger twins whereas the opposite was found for the older twins. The current findings may help to elucidate how the complex interplay between genetic and environmental factors contributes to stability and change in neuroticism.

INTRODUCTION

The current study investigates the longitudinal structure of neuroticism, using a behavioural genetic twin design, that can examine how genetic and environmental sources of variance influence stability and change. Neuroticism is an important broad high-order trait in the big five structure of personality (Eysenck & Eysenck, 1975; McCrae & Costa, 1997) and has been shown to be the strongest correlate of psychopathology (Kotov, Gamez, Schmidt, & Watson, 2010; Lahey, 2009; Ormel, Riese, & Rosmalen, 2012). Not surprisingly, neuroticism 'accounts' for a substantial proportion of current and lifetime comorbidity (Clark, 2005; Khan, Jacobson, Gardner, Prescott, & Kendler, 2005; Kotov et al., 2010). A substantial overlap has been found between genetic influences on neuroticism and common mental disorders (Hettema, Neale, Myers, Prescott, & Kendler, 2006). Thus, quantification of the genetic and environmental influences on both stability and change in neuroticism may contribute to our understanding of the association between neuroticism and mental disorders and the etiology of the latter as well.

Stability and change in both mean level and individual differences (differential stability) in neuroticism have been extensively studied (Bleidorn, Kandler, Riemann, Angleitner, & Spinath, 2009; Conley, 1984; Hopwood et al., 2011; Ormel, 1983; Roberts, Walton, & Viechtbauer, 2006; Watson & Walker, 1996). Although small decreases with time and age (maturation) have been found, longitudinal studies reported substantial mean-level stability during middle and late adulthood. Regarding differential stability, meta-analytic evidence has firmly established increasing stability across the life course until it reaches a peak in later adulthood but also decreasing stability with increasing time interval between measurement occasions (Fraley & Roberts, 2005). Recent studies not included in the meta-analyses, confirm these findings (Kandler et al., 2010; Lüdtke, Trautwein, & Husemann, 2009). Differential stability seems to asymptote at a stability of about .40 over more than 20 years (Ormel & Rijsdijk, 2000; Wray, Birley, Sullivan, Visscher, & Martin, 2007).

Less agreement exists regarding the mechanisms that underlie differential stability in neuroticism. Traditionally, neuroticism has been decomposed into a stable component that is due to genetic effects and a change component which is environmental. Over the last decades, evidence has grown that this may be overly simplistic. For example, decreasing genetic influences have been reported with age (Viken, Rose, Kaprio, & Koskenvuo, 1994). Three etiological theories have been proposed to explain differential stability in neuroticism. The genetic set-point hypothesis posits that genetic factors determine individual set points to which individuals will return after environmentally influenced, short term changes in personality scores (Carey, 2002). The genetic maturation hypothesis assumes that rank-order stability is exclusively mediated by genetic factors and suggests that significant environmental effects on personality traits mainly result from short-term influences and systematic as well as random measurement error (McCrae et al., 2000). The genotype-environment transaction hypothesis proposes that stability results from transactions between genetic and environmental factors contributing to estimates of both genetic and

environmental effects on phenotypic stability and change (Caspi, Roberts, & Shiner, 2005). Kandler and colleagues (2010) have referred to these three hypotheses in their 3-wave study on genetic and environmental influences on personality traits. Findings provided support for all three hypotheses. In line with the gene–environment transaction hypothesis, personality change was primarily caused by environmental factors. In addition, influences on long-term stability were exclusively genetic and innovation (occasion specific) effects decreased with age, providing support for the genetic maturation hypothesis and especially the genetic set-point hypothesis. However, attrition in their study was substantial (up to 79% in wave 3), and although the authors suggested that drop-out was at random, it seems that the focus of the attrition analyses was on differences between responders and non-responders at wave 1 (and less at wave 2 and 3). Consequently, it seems plausible that their attrition analyses did not provide information on differences between responders and non-responders regarding long-term stability and change. Individuals with a stable personality are therefore likely to be overrepresented in their sample, resulting in an overestimation of, in particular the genetic influences on, personality stability.

The goal of the present article is examining the longitudinal structure of neuroticism and reframing stability and change in the perspective of Trait (a stability factor) and State (a change factor), using a genetic extension of the Trait-State model. Genetic and environmental sources of the two components are estimated, which may contribute to our understanding of the etiology of mental disorders and their association with neuroticism. Moreover, we will investigate whether heritability of neuroticism decreases with age, thereby providing evidence for the increasing importance of the environment. Finally, we will be able to test whether the longitudinal structure of neuroticism is similar for older and younger twin pairs. Innovative of this study are the combination of a large sample, low attrition, and four assessment waves of neuroticism over a protracted period.

METHODS

Sample

The longitudinal sample for this study began with the 2,163 individual twins from female-female twin pairs who initially participated in the Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD). All twins were drawn from the population based Virginia Twin Register (Kendler, Neale, Kessler, Heath, & Eaves, 1992; Kendler & Prescott, 2006). Twins who were born between 1934 and 1974 and responded to the initial mailed questionnaire were eligible. The initial response rate was approximately 64%. Written informed consent was obtained before all face-to-face interviews, and verbal assent was obtained for all phone interviews. Zygosity was determined blindly by standard questions (Eaves, Eysenck, & Martin, 1989), photographs, and, when necessary, DNA (Spence et al., 1988). Eight twins were dropped due to unresolved zygosity classification. This resulted in a sample that consisted of N=597 complete and N=48 singleton monozygotic twins and N=433 complete and

N=47 singleton dizygotic twins. After the initial questionnaire, twins were approached and interviewed four subsequent times between 1988 and 1997. The mean number of months between the interviews was respectively 17.3 months, SD = 3.8 (wave 1-wave 2), 45.0 months, SD = 4.0 (wave 2-wave 3) and 31.5 months, SD = 6.8 (wave 3-wave 4). Cooperation rates across waves ranged from 85% to 92%. The first wave was a face-to-face interview, while the second, third and fourth waves were predominantly completed by phone. At the time the participants completed the first interview, they ranged in age from 18 to 54 (M = 29.3, SD =7.7). For the current study we included data from 1,125 twin pairs (57.3% monozygotic), both complete and incomplete cases. Missingness at the individual level was about 4% at w1, 15% at w2, 12% at w3 and 21% at w4, and due to non-response and attrition.

Neuroticism

Neuroticism was assessed in four of the available five waves. In the initial questionnaire, neuroticism items were part of 54 items included from the Eysenck Personality Questionnaire (EPQ). In the follow up waves 1, 3, and 4, 12 items from the shortened EPQ (Eysenck & Eysenck, 1975; Eysenck, Eysenck, & Barrett, 1985) were included as either part of the main interview (FF3 and FF4) or as a separate self-report questionnaire (FF1). For each occasion a composite score was created by summing the 12 binary items (no = 0 and yes = 1). These sum variables displayed increasing positive skewness across waves. To address possible assumption violations (e.g., multivariate normality), variables were modeled as ordinal. Due to the prohibitive number of estimated thresholds (12 per occasion); variables were reorganized into 5 ordered categories jointly. Although variables were treated as ordinal, the parameterization proposed in Mehta and colleagues (2004) was used. By fixing the first and second thresholds to 0 and 1 and estimating the remaining thresholds, the model can test change hypotheses using the normal continuous latent response variables (polychoric correlations). The test-retest stability (reliability) of neuroticism was respectively .63 (wave 1-2), .60 (wave 2-3) and .67 (wave 3-4).

Statistical analyses

Over the past decades, new methodologies have been developed enabling researchers to study longitudinal structures using a Trait-and-State model (Duncan Jones, Fergusson, Ormel, & Horwood, 1990; Kenny & Zautra, 1995; Ormel & Schaufeli, 1991). Using a Trait-State model, Trait variance can be disentangled from State variance. Whereas Trait variance reflects stable individual differences State variance represents occasion specific variation. The State component was further decomposed into both auto-regressive (short-term stability across one time interval) and innovation (occasional specificity) parameters. In addition, to solve the model it is necessary to assume that Trait and State component are statistically independent (Ormel & Rijsdijk, 2000). Here we use a behaviour genetic Trait-State model, in which measurement error is subsumed under (and thus confounded with) the environmental innovation component. In this specification, separate occasion-specific measurement errors

as described by Kenny and Zautra (1995) are not included in the model because these parameters are not identified.

We extend the latent Trait-State model to include genetic and environmental components in order to evaluate the extent to which observed phenotypic stability and change is determined by genetic and environmental effects. The model (Figure 1) includes: 1) genetic and environmental (non-)shared effects that influence the stable individual differences portion of neuroticism across the four time points as modeled by a common latent Trait factor, 2) genetic and environmental effects that have an effect on the occasion specific aspects of neuroticism through a State component consisting of genetic and environmental innovation (wave 1 - 4) and genetic and environmental auto-regression effects (wave 2 – 4). Whereas the relative contribution of genetic and environmental Trait influences are the same across waves, the contributions in the carry-over and innovation effects within the State component can differ between the subsequent waves. To examine differences between older and younger twins, the sample was split based on the mean age (29.3 years). This resulted in a sample of young twins (n =627) and a sample of older twins (n=405). For 23 twin pairs no information on age was available. Z-tests for independent samples were used to examine whether differences in proportions of explained trait and state variance differed significantly between the two age groups. Tests of whether proportions of trait and state variance significantly differed across waves (within each age group) were performed by means of one-sample z-tests.

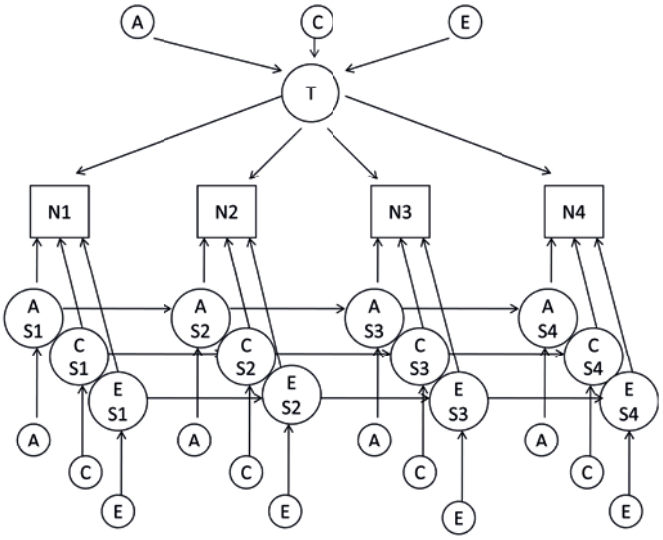

Figure 1. Schematic representation of the Trait-State model with genetic (A), shared-environmental (C) and non-shared-environmental (E) influences. Circles are latent variables, squares observed variables and triancles reflect the means at each time point. See Figure 2 for model specification. All exogenous variables (without single-headed paths pointing to them) have variance fixed at unity.

This genetic extension of the classic Trait-State model differs from the models used by Kandler and colleagues (2010), referred to in the introduction of this report, with regard to the auto-

regressive part of the model. Kandler links the latent true score variables by means of auto-regression. Consequently, trait variance can be transmitted through auto-regression. In our model, in contrast, the latent true score variables are linked through separate state variables. Although both Kandler's and our model are valid, the current model enables a stricter decomposition between auto-regressive and true trait variance.

Analyses were performed using the free OpenMx package developed within the R language (Boker et al., 2011). OpenMx offers a flexible structural equation modeling platform to specify and estimate longitudinal twin models using full-information maximum likelihood (FIML) estimation. With OpenMx, the additive genetic (A), shared environmental (C) and non-shared environmental (E) components are estimated using an iterative search process that finds parameter values that reproduce the observed MZ and DZ twin variance–covariance matrices as closely as possible. Comparisons of model fit were carried out to select a best-fitting model based on the Akaike information criterion (AIC; Akaike, 1987). The lower the AIC value, the "better" the overall model fits the data.

RESULTS

Descriptives

Descriptive statistics for the total sample are presented in Table 1. Mean values were based on the rescaled neuroticism scores. From wave 1 to wave 4, the mean neuroticism scores decline significantly ($p < .001$). Older and younger twins pairs did not differ with regard to changes in neuroticism ($p = .465$). Cross twin correlations are higher in MZ than in DZ twins, suggesting the presence of genetic influences (Eaves et al., 1989). Overall, correlations decline as intervals between time points increase.

Table 1. *Means, standard deviations and correlations between the four time points (MZ twins below and DZ twins above diagonal).*

		Twin 1				**Twin 2**					
		N1	**N2**	**N3**	**N4**	**N1**	**N2**	**N3**	**N4**		
		r	***r***	***r***	***r***	***r***	***r***	***r***	***r***	**Mean (SD)**	**N**
Twin 1	N1	1.00	.63	.58	.54	.13	.19	.21	.23	2.65(1.23)	430
	N2	.63	1.00	.70	.57	.01	.09	.08	.14	1.98(1.29)	370
	N3	.48	.54	1.00	.69	.05	.06	.11	.17	1.86(1.26)	402
	N4	.46	.54	.63	1.00	.09	.10	.09	.16	1.44(1.17)	361
Twin 2	N1	.34	.35	.33	.29	1.00	.56	.56	.43	2.74(2.01)	436
	N2	.32	.41	.33	.36	.62	1.00	.65	.59	2.01(1.27)	383
	N3	.25	.28	.31	.27	.52	.56	1.00	.69	1.86(1.32)	383
	N4	.19	.23	.23	.24	.52	.60	.65	1.00	1.50(1.26)	357
Mean (sd)		2.71 (1.14)	2.03 (1.25)	1.80 (1.18)	1.43 (1.17)	2.71 (1.19)	2.00 (1.30)	1.85 (1.24)	1.36 (1.15)		
N		607	539	545	499	598	533	557	483		

Model fitting results

Our goal was threefold: 1) to determine to what degree the longitudinal structure of neuroticism could be attributed to Trait versus State components, 2) estimate the relative contribution of additive genetic (A), shared environmental (C) and non-shared environmental influences (E) for the four subsequent waves and 3) examine whether the longitudinal structure of neuroticism was similar for older and younger twins.

Trait versus State components. First, we tested the full ACE Trait-State model including latent Trait and State factors derived from the four neuroticism waves (Figure 1). As proposed by Duncan Jones and colleagues (1990), factor loadings were constrained to be equal across waves. Although allowing factor loadings to vary at the different time points may improve the fit of the data, equality constraints across time are a key feature for the interpretation of the single common factor as being a stable characteristic of individual differences in neuroticism co-variation over time.

Subsequently, we compared the full ACE model with an AE model in which all shared environmental components were dropped from the model. Model fitting results and comparative fit statistics are reported in Table 2. In line with the literature, the fit of this model was not significantly worse than that of the full ACE model and the subsequent analyses were carried out using the more parsimonious AE model.

Model comparison of the AE Trait-State model with the AE Trait-only model and the AE Trait-State model with the AE State-only model suggested that the covariance pattern of the neuroticism data could not be explained by either a Trait-only or a State-only model (Table 2). Comparison of the AIC values indicated a better model fit of the AE Trait-State model. Figure 2 presents proportions of explained variance of the Neuroticism scores explained by the State and Trait components. Total Trait and total State variance together are 1. For reasons of clarity we have only reported on the standardized solution. Unstandardized path estimates and confidence intervals are available upon request. Additionally, all relative A, E, Trait and State components are presented in Table 3.

Genetic versus environmental influences. Second, we estimated respectively the contribution of genetic and environmental influences (Table 3). The contribution of genetic influences was somewhat smaller than that of non-shared environmental influences and decreased slightly over time (for the difference between the environmental and genetic component $p < .001$ at wave 4). Genetic and environmental influences on the Trait component did not differ significantly. Regarding the State component, the largest part was attributed to innovation of the non-shared environment (e.g., for wave 2-4, $p < .001$), except at wave 1, when a relatively substantial amount of variance was explained by genetic innovation. However, also at wave 1 the environmental contribution was significantly larger than the genetic contribution ($p = .003$). The autoregressive part of the State component was mainly genetic (for wave 1-2 and 2-3, $p < .001$) but declined from wave 3 to 4 ($p = .134$). Almost no State variance was explained by transmission of non-shared environmental effects. The decline in the autoregressive

component is in line with the differences in average elapsed time intervals between the subsequent assessments. The interval between wave 1 and 2 was shorter than between the other waves.

Table 2. *Model fitting results of the neuroticism scores.*

Model	**-2*LL**	**Df**	**AIC**		**Δ-2*LL**	**Δdf**	***p***
Total sample							
1. ACE Trait-State	19616.75	7447	4722.75	-	-	-	-
2. AE Trait-State	19624.18	7455	4714.18	2. vs. 1.	7.42	8	0.49
3. AE Trait	19780.42	7468	4844.42	3. vs. 2.	156.24	13	0.00
4. AE State	19713.09	7456	4801.09	4. vs. 2.	88.92	1	0.00
Old (n=405)							
5. AE Trait-State	7225.27	2784	1657.27	-	-	-	-
6. AE Trait	7291.77	2797	1697.77	6. vs. 5.	66.50	13	0.00
7. AE State	7300.60	2785	1730.60	7. vs. 5.	75.33	1	0.00
Young (n=627)							
8. AE Trait-State	11412.10	4278	2856.10				
9. AE Trait	11507.65	4291	2925.65	9. vs. 8.	95.55	13	0.00
10. AE State	11447.45	4279	2889.45	10. vs. 8	35.35	1	0.00

Age differences. For both age groups, the Trait-State model provided the "best" fit (Table 2). However, the groups did differ with regard to proportion of trait and state and with regard to the strength of the genetic and environmental influences. Figure 3a and 3b, and Table 3 present proportions of explained variance of the neuroticism scores. Overall, the increase across waves in the trait variance was smaller in younger than in older twins. Whereas the proportions of trait variance did not differ between the groups with regard to wave 1, the difference increased across waves, resulting in a significant larger trait component in older twins than in younger twins at wave 4 ($p = .023$). Also the decrease across waves in genetic variance was larger in older than in younger twins. Both in older and in younger twins, the genetic (respectively $p = .002$ and .014) and the environmental (respectively $p < .001$ and $p = .010$) contributions increased from wave 1 to wave 4. Importantly, the genetic sources of trait variance are larger than any of the genetic proportions in the state component in the younger age group (all p-values $< .001$), whereas the reverse was found for the older twins. For the older twins the environmental sources of trait variance (except at wave 1) are larger than the environmental proportions of the state component (p ranging from $< .001$ to .012).

Table 3. *Proportion of variance explained by the different factors of the Trait-State model fitted on the Neuroticism waves presented for the total sample and split for the two age groups.*

		Total G	Total E	Total Trait	Total State
Wave 1	Total	52	58	44	56
	Young	42	58	43	57
	Old	45	55	44	56
Wave 2	Total	42	58	58	42
	Young	46	54	59	41
	Old	40	60	55	45
Wave 3	Total	40	60	57	43
	Young	43	57	58	42
	Old	35	65	58	42
Wave 4	Total	45	65	63	37
	Young	39	61	59	41
	Old	28	72	66	34

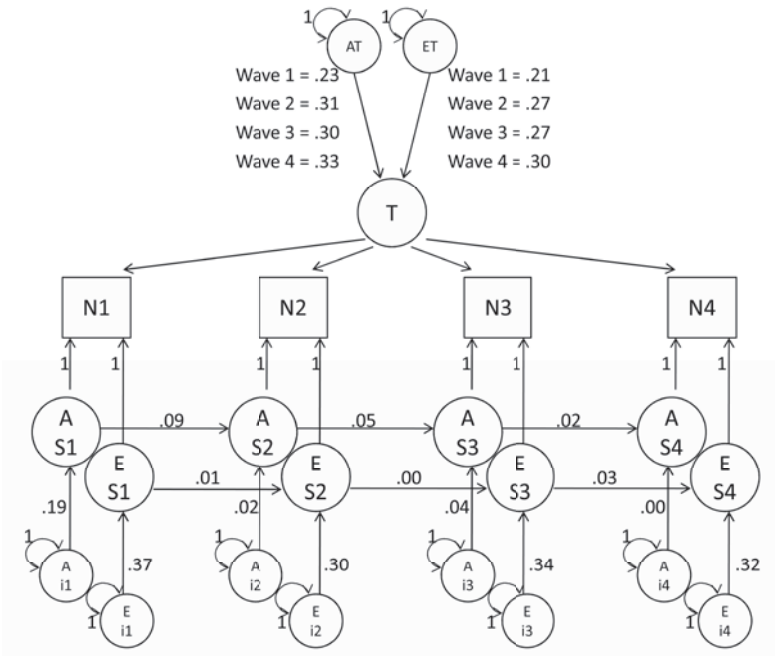

Figure 2. Total sample. Proportions of variance of the Trait-State model with genetic (A) and non-shared-environmental (E) influences.

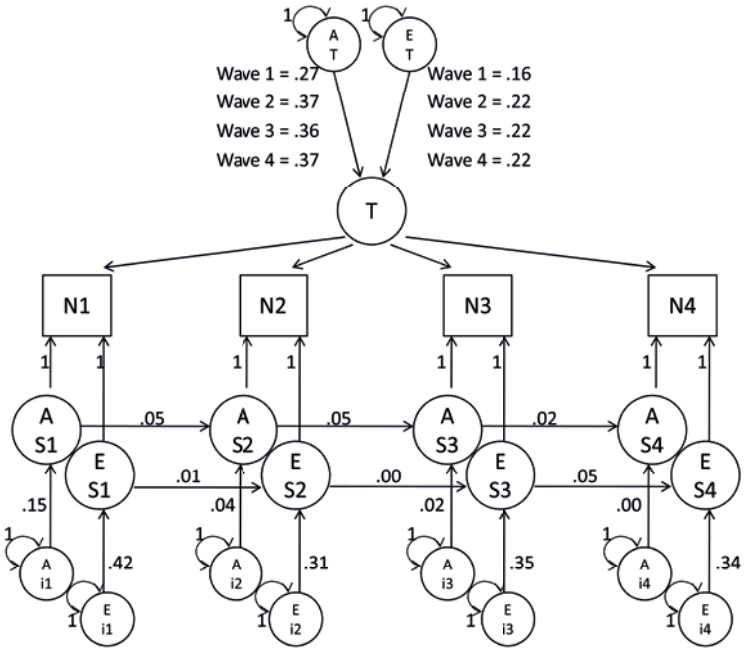

Figure 3a. Younger twins. Proportions of variance of the Trait-State model with genetic (A) and non-shared-environmental (E) influences.

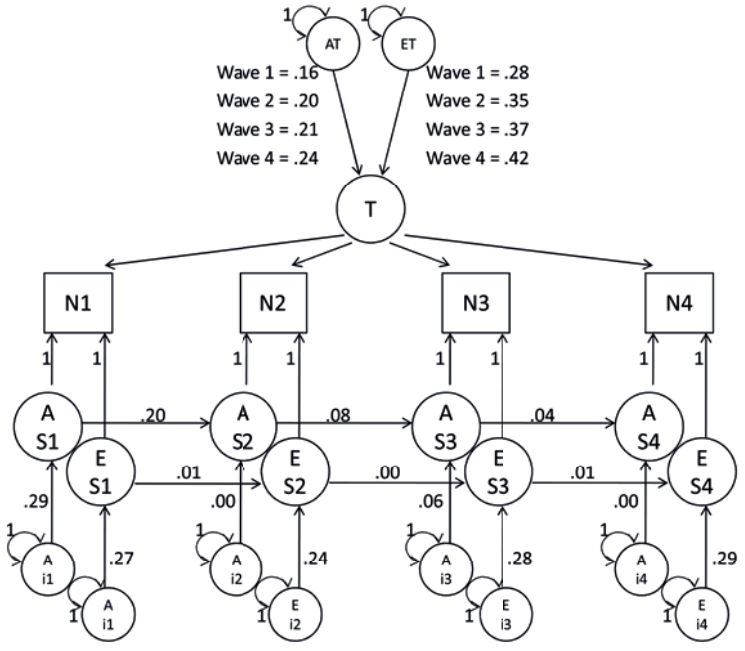

Figure 3b. Older twins. Model proportions of variance of the Trait-State model with genetic (A) and non-shared-environmental (E) influences

DISCUSSION

Combining a Trait-State approach with a genetically informative design, this study sought to extend our knowledge of the longitudinal structure of neuroticism. A Trait-only, a State-only and a Trait-State model were fitted to four waves of neuroticism data. Our results show that the Trait-State model fits the data well, indicating that longitudinal correlations have a Trait factor as well as a State component that includes both auto-regressive and innovative effects. Both the Trait and the State component were influenced by genetic and environmental effects, with - as expected - relatively more genetic variance in the Trait component. In our study, substantially more Trait variance could be accounted for by non-shared environmental effects than reported in two earlier studies (Kandler et al., 2010; Viken et al., 1994). Although the model held for both younger and older twins, the strength of the genetic and environmental influences differed, with more environmental Trait variance in the older twins.

Our findings are most consistent with the gene-environment transaction hypothesis. As postulated by Caspi and colleagues (2005) and further tested by Kandler and colleagues (2010) both continuity and change result from transactions between genetic and environmental factors. Moreover, our finding that stability of neuroticism increased across waves and with age, as well as the fact that the environmental component was larger in older than in younger twins, is in line with both 1) *social selection* (people select environments that correlate with their neuroticism) and 2) *social influence (*these environments produce experiences that influence neuroticism; Caspi et al., 2005). Our findings do not provide substantial support for the genetic maturation hypothesis. Although we found some evidence for new genetic influences (but not at the last wave), stability was not primarily influenced by new genetic factors. Limited evidence was also found for the genetic set-point hypothesis. Although our findings support the notion of a set point, the qualification that the set point has only genetic sources did not hold; we found substantial environmental influences, especially in the older twins.

This substantial environmental influence on the Trait component, in particular on older twins, is perhaps the most exciting finding. Traditionally it has been assumed that genetic influences are mainly reflected by stability whereas environmental influences affect change. Moreover, since heritability has traditionally been found to increase over time and across age in other fields of research (e.g., cognitive abilities), changes in heritability with regard to neuroticism have often been neglected. Our findings suggest that heritability of neuroticism tends to decrease. Only a few studies have pointed to the possibility of genetic influences on change and of (increasing) non-shared environmental influences on stability (Kandler et al., 2010; Viken et al., 1994). Our study convincingly confirms this possibility. Nearly half of the Trait variance has environmental origins. This can reflect childhood experiences (Eysenck & Eysenck, 1975), but may also confirm the suggestion that people select their own environment and therefore enhance continuity of their personality (Caspi et al., 2005; Krueger, Johnson, & Kling, 2006; Scarr & McCartney, 1983). Non-shared environmental effects

also contributed substantially to change. About a third of the variance in neuroticism scores could be explained by non-shared environmental innovation. Although this is in line with previous studies (e.g., Wray et al., 2007), caution should be used when interpreting these proportions. Both in our study and in the studies mentioned, non-shared environmental effects are confounded with time specific error variance. Additionally, in accord with Bleidorn and colleagues (2009), our results suggest that genetic effects are not exclusively involved in stability, but that new genetic effects may also influence change in neuroticism scores. This genetic innovation was substantial at the start of the study because it subsumes all genetic transmission effects which are not captured by the Trait component. Moreover, at the first wave autoregressive variance cannot be disentangled from innovation and statistically, all state variance is per definition included in the innovation component. Consequently, some caution is needed when comparing the genetic and environmental contributions at the first wave with the contributions at the subsequent waves.

Of the small auto-regressive component in our study, almost all of this effect was due to transmission of genetic variance. This suggests that 1) new non-shared environmental effects, although present, only influence neuroticism scores for a relatively short period of time and 2) new genetic effects; although small in magnitude, have the potential to influence neuroticism scores over a protracted period. In addition, the modest amount of environmental auto-regression, is consistent with Kendler and colleagues (2011), showing increasing divergence of scores for depression and anxiety symptoms (which are highly correlated with neuroticism) with increasing age in MZ twin pairs.

Not only the findings on environmental auto-regression, but also other aspects of our model can be interpreted in terms of psychopathology. Most important, our results show that an individual's level of neuroticism is not set in concrete but somewhat modifiable if the cumulative transactions of social selection and social influence that determine neuroticism can be turned around in a more adaptive direction. Given that neuroticism has been shown to be the strongest predictor of psychopathology the significant environmental influences on the neuroticism set-point and the change in neuroticism imply the possibility to modify neuroticism through preventive and treatment interventions.

Despite a variety of strengths, including the twin design, large epidemiologically representative sample, limited attrition, multiple waves, and long study period, the current study has limitations as well. Although our findings suggest age effects, to study age in more detail than we did by splitting the sample, time intervals between waves should be more equally distributed over the years. Related to the issue of unequal time intervals, is the drop in mean neuroticism scores across waves. Small drops with aging have often been reported in the literature, but the larger declines we found in our sample appear atypical. Some decline can probably be explained by test-retest effects and by age. In addition, whereas at the first time point a self report questionnaire was used, at all other time points an interview was administered. By developing a more complex statistical model than the already sophisticated

one currently being used, it might be possible to disentangle mode and interval length, and subsequently, to gain additional insight in mean-level changes. Future research might address this methodologically interesting, but highly complex issue. Additionally, by moving towards a more methodological approach, it might be possible to test whether and how (slight) modifications in the model would affect the current results. Testing alternative assumptions and constraints would provide information on the robustness of the current findings. For example, it would be interesting to compare our findings with results of a model as proposed by Kenny and Zautra (1995), in which a stationary constraint is imposed so that the contributions of Trait and State components are the same at each wave, and the stability of the autoregressive component is assumed to be the same over time. Nonetheless, given the aims of the current study (i.e., disentangling Trait and State components of neuroticism), and the limitations of a four wave design (i.e., under-identification of more complex models), such a methodological approach was beyond the scope and possibilities of our study.

Another limitation might be the assumption of uncorrelated genetic influences on the Trait and the State factors. From the perspective of most genetic studies, it seems reasonable to allow different genetic influences to correlate. However, the classic Trait-State model assumes that Trait and State components are independent. We feel that this is reasonable to assume since these forms of variance can have different genetic effects. The Trait factor covers the genetic influences which are stable across the total study period whereas the genetic influences on the State components are not immutable across the study period but time dependent (age, context). Finally, our sample included only female twins. Although we do not envision a priori why the longitudinal structure of neuroticism would be different in men, the current findings may not generalize to men.

In conclusion, the current study had the aim to disentangle the longitudinal structure of neuroticism and to estimate the genetic and environmental contributions to differential stability and change. In line with classic views on personality a substantial Trait component was found, but additionally, and confirming some more recent studies (Kandler et al., 2010; Ormel & Rijsdijk, 2000; Viken et al., 1994), a substantial State component was detected (including both innovation and some auto-regression). Remarkably, and new to the literature, the contributions of genetic and environmental influences on the Trait component were nearly equal, suggesting that the genetic set-point model of neuroticism is incomplete. All things considered, the data most strongly support the genotype-environment transaction model (Caspi et al., 2005). From this perspective, neuroticism is best seen as the result of two mutually supportive life-course dynamics; (1) social selection and (2) social influence. This insight provides heuristic tools for changing neuroticism, and hence vulnerability.

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3

Adolescent personality: Associations with basal, awakening and stress-induced cortisol responses

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Submitted

ABSTRACT

The purpose of the present study was to investigate the associations between personality facets and hypothalamic-pituitary-adrenal (HPA) axis functioning. Previous studies have mainly focussed on stress-induced HPA-axis activation. We hypothesized that other characteristics of HPA-axis functioning would have a stronger association with personality based on the neuroendocrine literature. Data (n = 343) was used from TRAILS (Tracking Adolescents' Individual Lives Survey), a large prospective cohort study of Dutch adolescents. We studied the association between facets of neuroticism, extraversion and conscientiousness and basal cortisol, the cortisol awakening response (CAR), and four measures of stress-induced HPA-axis activity. Basal cortisol levels were related to facets of all three personality traits. The CAR and stress-induced cortisol were not related to personality. Possibly due to its more trait-like nature, basal cortisol seems more informative than stress-induced cortisol, when investigating trait-like characteristics such as personality facets.

INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis is a key component in the body's neuroendocrine stress response, and its end product, cortisol, has been implicated in the transduction of psychosocial stress into psychopathology (Herbert, 1997; Susman, 1998). Functioning of the HPA-axis has become increasingly popular in the study of mechanisms underlying the development of psychopathology. Although the associations are complex, atypical HPA-axis functioning has been suggested to be related to psychopathology (e.g., Burke, Davis, Otte, & Mohr, 2005). Parallel to the study of cortisol and psychopathology is the study of personality and psychopathology. Similar to atypical HPA-axis functioning, atypical personality profiles have been posited to predispose to psychopathology (Khan, Jacobson, Gardner, Prescott, & Kendler, 2005; Kotov, Gamez, Schmidt, & Watson, 2010; Ormel, Rosmalen, & Farmer, 2004). Despite the complexity of the respective relationships, it seems clear that some people are at increased risk for psychopathology by virtue of their personality traits and/or HPA-axis functioning. The question that remains is whether and how functioning of the HPA-axis and personality are related to each other. The current study aims to investigate associations between various measures of HPA-axis functioning and personality facets during adolescence.

Measures of HPA-axis functioning

HPA-axis functioning can be studied at different levels. An important distinction can be made between basal levels of HPA-axis activity, and changes in HPA-axis activity. The *basal HPA-axis activity level* reflects the basal or resting metabolism of an organism (Hellhammer et al., 2007). Basal HPA-axis functioning can be operationalized as (a series of) cortisol sample(s) taken at a fixed moments during the day, for example in the morning (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

In healthy humans, HPA-axis activity follows a circadian rhythm (e.g., Fries, Dettenborn, & Kirschbaum, 2009; Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Cortisol, the main effector of the HPA-axis, is excreted in a pulsatile fashion (Young, Abelson, & Cameron, 2004) and concentrations start to rise during the second half of the night and reaches a peak in the early morning hours, to gradually decreases throughout the day (Dallman, 2000; Fries et al., 2009; Tsigos & Chrousos, 2002). Generally, basal HPA-axis functioning is operationalized as a single cortisol sample, measured at a fixed moments during the day (e.g., immediately after waking up in the morning). Cortisol concentrations are relatively stable when assessed at the same time throughout subsequent days (Hellhammer et al., 2007), and have a substantial genetic component (.62; Bartels, Van den Berg, Sluyter, Boomsma, & de Geus, 2003). Basal HPA-axis functioning is therefore suggested to be a trait-like characteristic.

In contrast, HPA-axis reactivity is an indicator of the sensitivity of the HPA-axis to specific situations (Hellhammer et al., 2007). The HPA-axis plays a crucial role in preparing the body for performing a specific task (Koolhaas et al., 2011), in other words, changes in HPA-axis activity

might be an indicator of the amount of energy or effort an individual is willing or able to invest in performing the task, more than perceived stress. HPA-axis reactivity can be studied in terms *of cortisol awakening responses (CAR),* or in terms of *cortisol responses induced by a (social) stress task*. The *cortisol awakening response (CAR)* reflects HPA-axis reactivity to the anticipated stress load of the upcoming day (Fries et al., 2009; Hellhammer et al., 2007). The CAR has generally been operationalized as the area under the cortisol curve with respect to the increase (AUCi) of the various assessments from wakening up to an hour after wakening (Pruessner et al., 2003; although alternative methods have also been proposed, e.g., Adam, 2006; Adam & Kumari, 2009), during the first half hour of which cortisol concentrations increase sharply (Kudielka et al., 2004). The CAR has a modest heritable component (e.g., .40 -.48, Wüst, Federenko, Hellhammer, & Kirschbaum, 2000; .52, Riese, Rijsdijk, Rosmalen, Snieder, & Ormel, 2009) for the increase in the first hour after awakening, and might therefore be considered more state-like than basal HPA-axis functioning.

In addition to CAR, changes in HPA-axis functioning can also be studied in terms of responses to stress, for example during a social stress task. Following the same argument as for the CAR, that changes in HPA-axis activity reflect an individuals' physiological preparation, *task-induced HPA-axis reactivity* reflects the extent to which an individual physiologically invests in performing a certain task (Koolhaas et al., 2011; Sapolsky, Romero, & Munck, 2000), thus might be an indicator of the amount of energy or effort an individual needs for performing the task, such as the Trier Social Stress Test (Benschop et al., 1998; Kirschbaum, Pirke, & Hellhammer, 1993), more than perceived stress. Task-induced HPA-axis reactivity is operationalized as the increase in cortisol concentrations from resting, usually measured prior to the task, compared to during the task. It is often calculated as a difference score, or as the residual of cortisol during the task regressed on resting cortisol (Burt & Obradović, 2012). For measurement of HPA-axis reactivity it is important to keep in mind that there is a delay of approximately 20 minutes between the onset of HPA-axis activity and detectability of increases in salivary cortisol (Kirschbaum & Hellhammer, 1992). This means that saliva samples do not need to be taken during the task, but can be taken immediately after the task. Heritability of stress-induced cortisol has also been found to be rather low (.33, Federenko, Nagamine, Hellhammer, Wadhwa, & Wüst, 2004). Nonetheless, this heritability of stress-induced cortisol has been found to increase substantially with repetition of the stressor, suggesting that whereas first-time stress-induced cortisol reflects a state characteristic, habituation to the task may be more trait-like (Federenko et al., 2004). Moreover, this seems to indicate that whereas the (empirical) basis for basal cortisol as a trait characteristic is substantial, CAR and stress-induced cortisol are probably not exclusively state-like.

Although almost all studies into the association between personality and stress-induced HPA-axis functioning have focussed on stress-induced HPA-axis reactivity, HPA-axis reactivity may not be the most informative measure of stress-induced HPA-axis functioning (Koolhaas et al., 2011). Research in rats showed that sexual behaviour elicited the largest increase in cortisol,

not an adverse stimulus (Koolhaas, de Boer, de Ruiter, Meerlo, & Sgoifo, 1997). This suggests that HPA-axis reactivity is indeed primarily a marker for energy mobilization, and not stress, but effort related (Koolhaas et al., 2011; Sapolsky et al., 2000). Moreover, when investigating HPA-axis responses to behaviours which differed in perceived stress (winning versus losing a fight, naïve versus experienced swimming), the increase in cortisol (HPA-axis reactivity) was the same, whereas rats differed in recovery of the HPA-axis after the task (i.e., the decrease in cortisol). These findings suggest that recovery rate is a more informative index of stress than reactivity (Koolhaas et al., 2011; Nederhof et al., Submitted), and thus, that recovery after stress might be an interesting cortisol index to study in addition to the more frequently studied reactivity.

The *recovery of the HPA-axis after a task* is determined by the strength of the negative feedback loop and might reflect perceived control over, or perceived stress in a specific situation (Koolhaas et al., 2011; Sapolsky et al., 2000). HPA-axis recovery can be operationalized as the decline in cortisol concentrations from during the task to after the task and can be calculated as either a difference score, a residual score, or a slope when more than one recovery measure was taken (Burt & Obradović, 2012). As salivary cortisol concentrations reflect HPA-axis activity 15 min earlier, a recovery measure should be taken approximately 40 min after the end of the task.

Although not directly an index of change, another interesting measure of HPA-axis functioning in the context of stress may be anticipation. *Anticipatory HPA-axis activity* reflects an individual's arousal in expectation of an event. Anticipatory HPA-axis activity can be operationalized as cortisol concentration preceding an event, for example after coming in to the lab before the start of the experiments. Apparently, in humans, HPA-axis activity in expectation of an event with unknown content is associated with mental health. Mikolajczak and Luminet found that lower anticipatory cortisol was associated with higher scores on a resilience questionnaire (Mikolajczak & Luminet, 2008). Likewise, although not tested for significance, results from Young and colleagues suggested that anticipatory cortisol concentrations were lower in healthy participants compared to participants with affective and/or anxiety disorders (Young et al., 2004). In the present study we will explore whether anticipatory HPA-axis activity is also associated with personality.

A final measure of HPA-axis functioning we will investigate is the total cortisol output during the stress task (STAUCg). In contrast to measures of stress-induced cortisol, emphasizing changes over time and, in particular in the case of stress reactivity, sensitivity of the system, *total HPA-axis activity during a task* primarily reflects the magnitude of a response, including both sensitivity (the difference between the single measurements from each other) and intensity (the distance of these measures from ground; Fekedulegn et al., 2007; Pruessner et al., 2003). Total cortisol output during a task can be operationalized as the area under the curve with respect to the ground (AUCg); the sum of changes in cortisol concentrations (Pruessner et al., 2003) superimposed on the diurnal rhythm. The stress task AUCg (STAUCg) can be seen as a measure of stress-induced cortisol that is influenced both by state and trait components.

Measures of Personality

Whereas research has barely focused on different measures of HPA-axis functioning, personality literature has traditionally distinguished various facets, or traits. The focus on different personality traits has resulted in several slightly different three and five-factor measures (De Raad & Perugini, 2002).Together, the (three or five) factors are widely accepted as facilitating a comprehensive and detailed picture of an individual's personality profile. The broad factors of neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness have appeared to explain most of the common variance among normal personality traits (Digman, 1990). For the current study we have focused on facets of neuroticism, extraversion and conscientiousness, the three personality traits that have been most consistently linked to psychopathology (Kotov et al., 2010). With regard to neuroticism we distinguish between vulnerability (i.e., general susceptibility to stress), angry/hostility (i.e., tendency to experience anger and related states such as frustration and bitterness) and impulsivity (i.e., tendency to act on cravings and urges rather than reining them in and delaying gratification). Two facets of extraversion are distinguished: assertiveness (i.e., social ascendancy and forcefulness of expression) and excitement seeking (i.e., need for environmental stimulation). Regarding conscientiousness, we will focus on self-discipline (i.e, capacity to begin tasks and follow through to completion despite boredom or distractions; Costa Jr & McCrae, 1992).

Associations between personality and HPA-axis functioning

Given the various facets of both HPA-axis functioning and personality, it should not be surprising that there is no easy-to-view picture of the association between the two. Some hypotheses regarding the associations might be formulated based on both theoretical arguments and previous research. First, personality has traditionally been assumed to be a trait-like characteristic. Although a recent behavioural genetic study has provided evidence for both a state component and a trait component in neuroticism (Kandler et al., 2010; Laceulle, Ormel, Aggen, Neale, & Kendler, In press) the substantial heritability of personality (Bouchard & McGue, 2003; Heath, Neale, Kessler, Eaves, & Kendler, 1992), suggests that all facets of personality traits have a stronger relation with trait aspects of HPA-axis functioning compared to state aspects. Consequently, it seems plausible that personality traits have the strongest association with trait-components of HPA-axis functioning (basal cortisol and to some extent also STAUCg).

Surprisingly, trait aspects of HPA-axis functioning have only incidentally been studied in relation to personality. In only one published study the association between basal cortisol and personality was investigated. Using a sample of 81 male and female students, Schommer and colleagues found that basal cortisol did not distinguish between subjects with high or low scores on either extraversion or neuroticism (nor did they find an associations for psychoticism, a third trait assessed in their study (Schommer, Kudielka, Hellhammer, & Kirschbaum, 1999). To the best of our knowledge, no studies have been performed on associations between

stress task AUCg and personality. Nonetheless, a study on associations between cognitive 'personality' traits and cortisol stress responses showed that in particular situation-specific cognitive traits (e.g., anticipatory cognitive appraisal) explained a substantial amount of variance in STAUCg (up to 35%; Gaab, Rohleder, Nater, & Ehlert, 2005). More general cognitive 'personality' traits (e.g., self-concept of own competence) were only weakly related to STAUCg (up to 8%). The authors suggest that situation-specific factors are more interesting to study in the context of a stress task than broader personality traits, possibly because they have comparable conceptual levels.

With regard to the more state-like aspects of HPA-axis functioning, some studies have assessed associations between personality traits and both cortisol awakening response and stress reactivity. Interestingly, all studies examining personality and cortisol awaking response have focused on neuroticism, whereas no studies seem to have assessed associations with other personality traits. The focus on neuroticism might be a result of the presumed link between neuroticism and low tolerance for stress or aversive stimuli (e.g., Norris, Larsen, & Cacioppo, 2007). Nonetheless, research into associations between neuroticism and cortisol awakening responses has resulted in inconsistent findings. Although most studies reported no significant associations (Chan, Goodwin, & Harmer, 2007; Riese et al., 2009; Wirtz et al., 2007), others found that individuals who scored extremely high on neuroticism had a higher CAR than individuals with an extremely low neuroticism(Portella et al., 2005; Schommer et al., 1999).

Without doubt, most research has been performed on the association between personality traits and reactivity to a stress task. Some studies did not find any association (Kirschbaum, Bartussek, & Strasburger, 1992; Schommer et al., 1999). For example, although Kirschbaum and colleagues examined many different personality traits, investigated with a number of questionnaires (i.e., the Eysenck personality Questionnaire, the Zuckerman Sensation Seeking Scale and the Strelau Temperament Inventory) no significant correlation was observed between stress reactivity and any of the personality measures studied. (Pruessner et al., 1997) reported negative associations between reactivity and facets of extraversion and conscientiousness, but only after data aggregation. Other studies reported associations between high levels of extraversion and a blunted cortisol response to stress (Kirschbaum et al., 1995; Oswald et al., 2006) or to elevated cortisol responses (LeBlanc & Ducharme, 2005). Similarly, high levels of neuroticism have been associated both with increased responses (Habra, Linden, Anderson, & Weinberg, 2003; Houtman & Bakker, 1991) and with blunted cortisol responses (LeBlanc & Ducharme, 2005; Oswald et al., 2006; Phillips, Carroll, Burns, & Drayson, 2005). With regard to conscientiousness, associations seem to be a bit more consistent, either no consistent association was found (e.g., (Oldehinkel, Hartman, Nederhof, Riese, & Ormel, 2011; Oswald et al., 2006) or higher conscientiousness was related to enhanced cortisol responses (Garcia-Banda et al., 2011; Oldehinkel et al., 2011). Given our earlier argument that HPA-axis reactivity reflects effort, it may be plausible that the previously reported inconsistent findings between

stress reactivity and personality traits (that is, the positive as well as the negative associations that have been reported for various traits) mainly reflect some fluctuation around the non-significant relation between personality and stress-induced HPA-axis reactivity.

Current study

In this project we investigated the associations between HPA-axis functioning and personality in a large population based sample of adolescents. In contrast to previous studies, we included various aspects of HPA-axis functioning as well as various facets of broader personality traits. Measures included were three different, but often studied aspects of HPA-axis functioning (basal cortisol, CAR, and reactivity to a stress task). Basal cortisol was operationalized as cortisol concentration at awakening. In addition to basal cortisol, the CAR and reactivity, anticipation and recovery elicited by a social stress task and STAUCg were included because those have been proposed as highly informative (Koolhaas et al., 2011; Pruessner et al., 2003), but have never been reported in the context of personality. Personality characteristics under study were facets of neuroticism, extraversion and conscientiousness, the three personality traits that have been consistently linked to psychopathology (Kotov et al., 2010).

In a large sample of adolescents, we tested the hypothesis that trait aspects of HPA-axis functioning, basal cortisol and possibly STAUCg, are stronger related to personality than the more state-like aspects of HPA-axis functioning, the CAR and stress task induced anticipation, reactivity and recovery. Consequently, we hypothesize that none of our personality facets is substantially related to stress reactivity. With regard to the personality facets under study, we expect that facets of neuroticism show stronger associations with basal cortisol than facets of extraversion and conscientiousness. However, given the previously reported non-significant association between basal cortisol and either extraversion or neuroticism (Schommer et al., 1999), it might be that only some, but not all, facets of neuroticism are related to basal cortisol. In particular the neuroticism facet 'vulnerability' is hypothesized to be related to basal cortisol, given the previously mentioned presumed link with sensitivity to stress (e.g., Norris et al., 2007).

METHODS

Sample

Data were used from the TRacking Adolescents' Individual Lives Survey (TRAILS), a large prospective cohort study of Dutch adolescents, who are followed biennially or triennially from 11 to at least 25 years of age (Ormel et al., 2012). The present study involves data from the third assessment wave, which ran from September 2005 to December 2007. At wave 1, 2230 pre-adolescents (50.8% girls) enrolled in the study (response rate 76.0%) of whom, 1816 (response rate 81.4%, 45.3% girls) participated in wave 3. At wave 3, the mean age was 16.13 years (SD = 0.59). A detailed description of the sample selection, procedures and methods can be found in de Winter and colleagues (de Winter et al., 2005).

During T3, 744 of the 1816 adolescents participating at wave 3 were invited to participate in a series of experiments in addition to the usual assessments. 715 (96.1%) agreed to do so. Adolescents with an increased risk of mental health problems had a greater chance of being selected for the experimental session. Increased risk was defined based on T1 temperament (high frustration and fearfulness, low effortful control), lifetime parental psychopathology, and environmental risk (living in a single-parent family). In total, 66.0% of the sample had one of the above-described risk factors; the remaining 34.0% were selected randomly from the total TRAILS sample (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). A previous study in the same sample by Bouma and colleagues (Bouma et al., 2009) on the effects of gender, menstrual phase and oral contraceptive use indicated that the use of oral contraceptive affects the cortisol awakening response as well as responses to the social stress test. Moreover, HPA-axis functioning in girls using oral contraceptives was so severely distorted (i.e., these girls did not show any cortisol response) that we couldn't consider oral contraceptives as a simple confounder. Therefore, these girls, as well as girls with missing data on oral contraceptive use were excluded from all analyses (n =126).

Other reasons for exclusion were smoking and use of coffee in the 2 h before the behavioural experiments (n = 4) as well as the use of steroid containing medication and SSRI's (n = 24). Further reduction of the sample was due to completely (n = 48) and partly (n = 170) missing cortisol samples and personality data. Final analyses were performed on complete cases (n = 343).

Procedure

TRAILS participants filled out questionnaires at school, in the classroom, supervised by one or more test assistants. In addition, a subsample of adolescents (see above) were invited to participate in the experimental session. The experimental session consisted of a number of different challenges, including orthostatic stress (from supine to standing), a spatial orienting task, a gambling task, a startle reflex task, and a social stress test; preceded and followed by a 40-min period of rest. For the current study we focussed on the social stress task. During the experimental challenges we assessed participants' psycho-physiological responses (cardiovascular, cortisol, and subjective experiences). Measures that were used in the present study are described more extensively below. The experimental sessions took place in sound proof rooms with blinded windows at selected locations in the participants' residence towns. The total session lasted about 3.5 hr and started between 8:00 and 9:30 a.m. (morning sessions, 50%) or between 1:00 and 2:30 p.m. (afternoon sessions). Adolescents were asked to refrain from smoking and from using coffee, milk, chocolate, and other sugar-containing foods in the 2 h before the session. At the start of the session, the test assistant explained the procedure and administered a short checklist on current medication use, oral contraceptives (OC), menstrual cycle, quality of sleep, and physical activity in the last 24 h. The protocol was approved by the Central Committee on Research Involving Human Subjects.

The Social Stress Test. This test was the final challenge of the experimental session. It involves a standardized protocol including public speaking and mental arithmetic, inspired by the Trier Social Stress Task (Kirschbaum et al., 1993), for the induction of moderate performance-related social stress. The TSST has been found to elicit significant changes in heart rate and in the HPA-system (Benschop et al., 1998). The participants were instructed to prepare a 6-min speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged on content of speech as well as on use of voice and posture, and rank-ordered by a panel of peers after the experiment. The participants had to speak continuously for the whole period of 6 min. The test assistant watched the performance critically, and showed no empathy or encouragement. The speech was followed by a 3-min interlude in which the participants were not allowed to speak. After the interlude, participants were instructed to subtract 17 repeatedly, starting with 13,278. This difficult task was meant to induce a sense of uncontrollability. Uncontrollability was further provoked by negative feedback by the test assistant, including remarks such as, "No, wrong again, begin at 13,278", "Stop wiggling your hands" or "You are too slow, be as fast as possible, we are running out of schedule".

Measures

Personality facets. The NEO-PI-R (Costa Jr & McCrae, 1992; Hoekstra, Fruyt, & Ormel, 2003) is a 240-item personality questionnaire which measures 30 personality facets, a selection of which were assessed in our study. For the present analyses we included all scales that were assessed in the TRAILS study: angry/hostility, impulsiveness and vulnerability (all facets of neuroticism), assertiveness and excitement seeking (both facets of extraversion) and self-discipline (a facet of conscientiousness). All scales consisted of eight items, which could be scored on a 5-point scale ranging from 1 = totally disagree to 5 = totally agree. Internal consistency (Cronbach α) ranged from .51 (impulsivity) to .77 (vulnerability).

HPA-axis functioning. To collect data on basal and awakening cortisol, participants received a verbal and written instruction to collect saliva at home immediately after waking up as they were still lying in bed (CM1; awakening/basal) and 30 minutes after awakening (CM2; awakening + 30), using the Sarstedt Salivette device (Nümbrecht, Germany). Directly after sampling, saliva samples were stored by participants in their freezer. We assessed HPA-axis responses towards the GSST by four cortisol samples (referred to as CE1, CE2, CE3 and CE5). There is a delay of approximately 20 min between the production of cortisol by the adrenal glands and the detectability of representative levels of cortisol in saliva. CE1 (pre-experiment), reflecting cortisol levels induced by anticipation stress, was taken at the start of the experimental session. CE2 (pre-stress) was collected just before the GSST, reflecting HPA axis activity 20 min earlier, when the participants filled out a rating scale, not related to the present study, and is considered a pre-test measure. CE3 (stress, speech) was collected directly after the end of the GSST and reflects cortisol levels during speech. CE4 (stress, arithmetic)

was collected 20 minutes after CE3 and reflects cortisol levels immediately after the GSST. CE5 (post-stress), collected 40 min after the end of the GSST reflects post-test cortisol levels.

After the experimental session, the samples were placed in a refrigerator at 4°C, and within a few days stored at -20°C until analysis. All samples were analyzed with the same reagent, and all samples from a participant were assayed in the same batch. Cortisol was measured directly in duplicate in 100 ml of saliva using an in-house radioimmunoassay applying a polyclonal rabbit cortisol antibody and 1,2,6,7 ^{3}H cortisol (Amersham, Arlington Heights, IL) as the tracer. After incubation for 30 min at 60°C, the bound and free fractions were separated using activated charcoal. The intra-assay coefficient of variation was 8.2% for concentrations of 1.5 nM, 4.1% for concentrations of 15 nM, and 5.4% for concentrations of 30 nM. The inter-assay coefficients of variation were 12.6%, 5.6%, and 6.0%, respectively. The detection border was 0.9 nM. Missing samples were due to detection failures in the lab (60%) or insufficient saliva in the tubes (40%). Cortisol levels above 5 SD of the mean were considered outliers and recoded into missing values.

Other Variables Experiment time, sex and habitual smoking were included as potential confounders of the associations under study. Smoking was assessed by questions on past and current smoking in a questionnaire which was filled out at school, on average 3.07 months (*SD* = 5.12) before the experimental session. We distinguished between non-smokers (n = 376) and habitual smokers (i.e., at least one cigarette a day, n = 123).

Statistical analyses

All analyses were performed in SPSS (Version 18.0). We first calculated descriptive statistics of the variables used in this study. Differences between boys and girls were tested by means of t-tests.

The standardized score of CM1, the cortisol measure immediately after awakening, was used as a measure of basal HPA-axis activity. With respect to awakening responses we subtracted CM1 from CM2 (when only two measures are available, calculating the formula proposed by Pruessner comes down to subtracting cortisol at awakening from cortisol 30 minutes after awakening, a method that has consistently been used in different studies; (Pruessner et al., 2003). Anticipation to the experimental session, reactivity to the GSST and recovery from the GSST were used as indices of stress-induced HPA-axis functioning. Anticipatory HPA-axis activity was operationalized as the first cortisol sample (CE1) taken at the start of the experimental session, approximately 1h before the start of the GSST. Reactivity and recovery were calculated by saving the standardized residuals of regression analyses: for reactivity, stress task cortisol (CE3, for most participants the highest cortisol level) was predicted by the pre-test measure (CE2), for recovery, post-test cortisol (CE5), was predicted by the task measure (CE3). Standardized residuals are commonly used in studies on stress reactivity and are the residuals divided by an estimate of their standard deviation. Similar to normal z-scores, they have a mean of 0 and a standard deviation of 1. Scores reflect the distance to the regression line and can consequently be used as a measure of change, that is,

positive scores represent relatively high HPA-axis activation compared to other adolescents (Burt & Obradović, 2012). Finally, the area under the curve with respect to the ground of the social stress task (STAUCg), reflecting total cortisol output during the test, was calculated using the following formula for AUCg recommended by Pruessner and colleagues ((Pruessner et al., 2003); ((CE3+CE2)*12.5) + ((CE4+CE3)*10) + ((CE5+CE4)*10). Basal cortisol, the CAR, anticipation and STAUCg scores were standardized into Z-scores. Standardized residuals are already similar to normal z-scores, they have a mean of 0 and a standard deviation of 1.

Using Fisher's Z-test we compared the bivariate correlation coefficients of cortisol measures and personality traits between boys and girls. If no consistent sex differences were found we would perform further analyses for boys and girls together. Subsequently, associations between HPA-axis functioning and personality traits were assessed in more detail by means of partial correlations. Smoking, sex and experiment time were included as covariates. Analyses were performed on complete cases (n = 343). Effects were marked as significant if $p \leq .05$ (two tailed).

Finally, we run three additional analyses with alternative operationalizations of cortisol measures. For CAR, we examined whether associations with personality traits were the same when CAR was operationalized as standardized residual (suggested as the most reliable operationalization for stress-induced reactivity (Obradović, Bush, Stamperdahl, Adler, & Boyce, 2010), instead of the commonly used difference score (Pruessner et al., 2003). For reactivity and recovery, we examined whether associations with personality traits were the same when they were assessed operationalized as difference score instead of standardized residuals.

RESULTS

Descriptive Statistics

Means and standard deviations of all variables are reported in Table 1. Boys were higher on assertiveness, excitement seeking, cortisol levels prior to (CE2) and during (CE3) the stress task, and with regard to reactivity to the stress task. Girls were higher with respect to vulnerability, impulsivity, cortisol levels 30 minutes after awakening and recovery after the stress task. They were also slightly higher on basal cortisol. A detailed description of cortisol responses to awakening and social stress in our sample (e.g., with regard to gender differences) can be found in Bouma and colleagues (Bouma et al., 2009). Bivariate correlations between single cortisol measures and personality traits are reported in Table 2.

Using Fisher's Z-test we compared all the bivariate correlation coefficients for boys with the correlation coefficients for girls. Significant differences were only found for three of the correlation coefficients. Correlations between respectively assertiveness and anticipation (Z = 1.99, p = .047), assertiveness and STAUCg (Z = 3.14, p = .002), and self-discipline and recovery (Z = 1.97, p = .049) were slightly stronger in girls than in boys. All other 33 differences in correlations were non-significant. Consequently, further analyses were performed for boys and girls together.

Table 1. *Descriptive statistics.*

	N	Mean (SD)	Sex differences
Vulnerability (N-facet)	502	2.37 (.52)	t(1, 500) = 6.76, ***p* = .000**
Impulsivity (N-facet)	502	2.90 (.46)	t(1, 500) = 2.02, ***p* = .044**
Angry/ hostility (N-facet)	502	2.45 (.53)	t(1,500) = - .79, *p* = .431
Assertiveness (E-facet)	502	3.02 (.55)	t(1, 500) =- 2.25, ***p* = .025**
Excitement seeking (E-facet)	502	3.54 (.51)	t(1, 500) =- 4.85, ***p* = .000**
Self-discipline (C-facet)	502	3.26 (.54)	t(1, 500) = .02, *p* = .987
Awakening (CM1)	417	7.82 (4.22)	t(1,412) = 1.98, ***p* = .049**
Awakening +30 min (CM2)	417	13.34 (5.55)	t(1,412) = 2.84, ***p* = .005**
Pre-experiments (CE1)	504	5.07 (4.33)	t(1,502) = - .89, *p* = .889
Pre-stress task (CE2)	506	3.67 (4.02)	t(1,504) = - .81, *p* = .421
During stress task (CE3)	513	4.83 (4.16)	t(1,511) = - 2.77, ***p* = .006**
Post-stress task (CE4)	506	4.72 (4.25)	t(1,504) = - 1.08, *p* = .280
20 min post-stress task (CE5)	505	3.93 (3.57)	t(1,503) = - .15, *p* = .879
Basal (Zscore CM1)	417	0 (1)	t(1,412) = 1.98, ***p* = .049**
CAR (CM2-CM1)	403	0 (1)	t(1,398) = 1,24, *p* = .216
Anticipation (Zscore CE1)	504	0 (1)	t(1,502) = - .89, *p* = .466
Reactivity (SR CE3 on CE2)	504	0 (1)	t(1,502) = - 3.52, ***p* = .000**
Recovery (SR CE5 on CE3)	505	0 (1)	t(1,503) = 3.05, ***p* = .002**
STAUCg (Zscore)	352	0 (1)	t(1,350) = .01, *p* = .998

Note. Basal and anticipation are the standardized values of CM1 and CE1. All cortisol measures are in nmol/l. N = neuroticism; E = extraversion, C = conscientiousness; CM = cortisol concentration in the morning; CAR = cortisol awakening response; CE= experimental cortisol concentration; STAUCg = stress task area under the curve with respect to the ground; SR = standardized residuals.

Table 2. *Correlations between single cortisol measures and personality traits.*

	Vulnera-bility	Impulsive-ness	Angry Hostility	Assertive-ness	Excitement Seeking	Self Discipline
CM1	**.237***	**.173***	.053	**-.135***	-.029	**-.214***
CM2	**.148***	.066	.032	-.088	-.029	**-.196***
CE1	-.047	-.051	.053	.052	-.016	.015
CE2	-.086	-.064	.016	.082	.039	.064
CE3	-.062	-.006	-.044	-.006	.058	.023
CE4	-.012	-.011	-.024	-.042	.006	.000
CE5	-.009	.059	.002	-.007	.001	-.034

Personality traits and cortisol responses to awakening and stress

Analyses presented were performed on complete cases. However, it should be noted that result of analyses excluding cases pairwise showed the same picture. Partial correlations

between personality traits and cortisol responses are reported in Table 3.

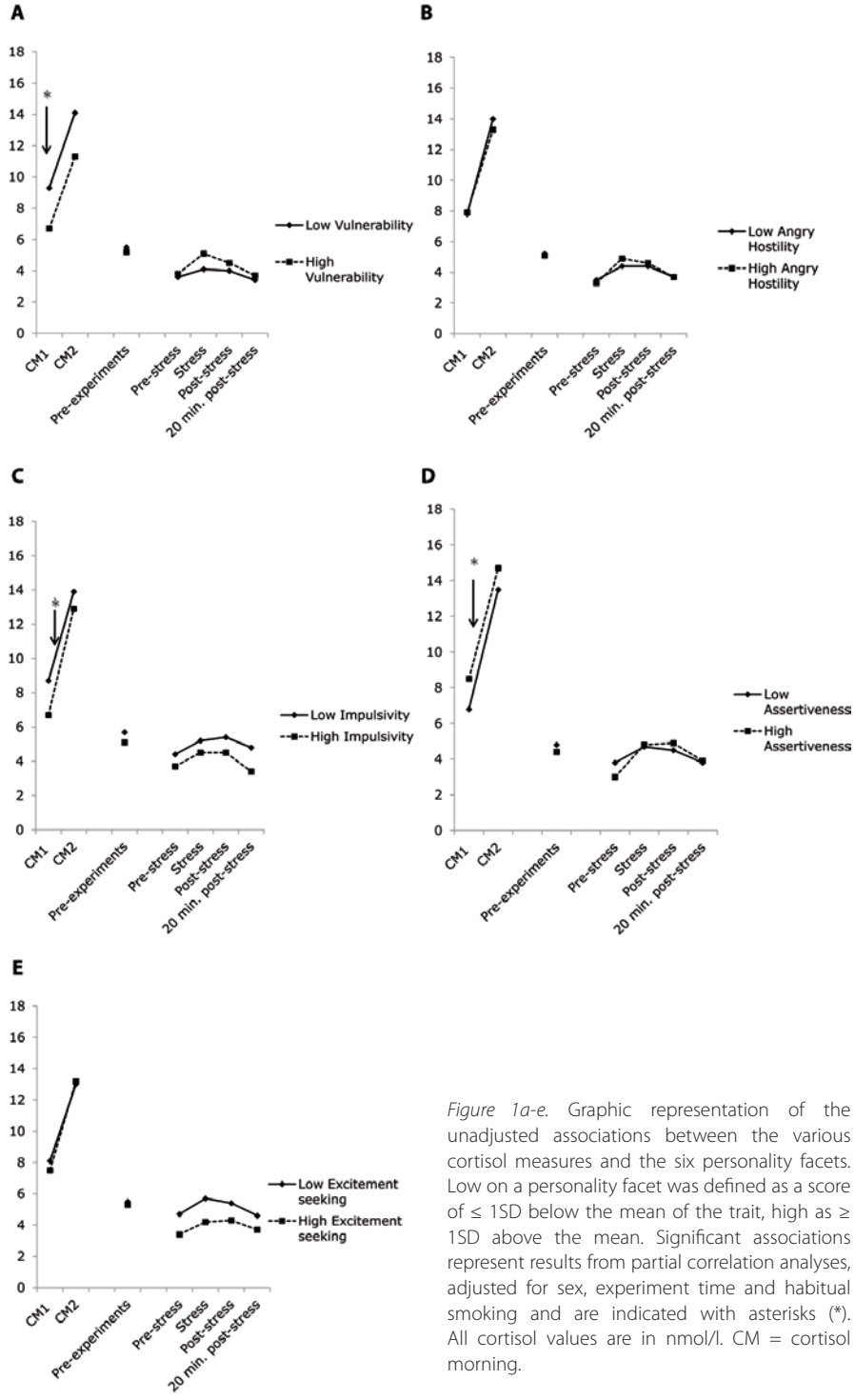

Figure 1a-e. Graphic representation of the unadjusted associations between the various cortisol measures and the six personality facets. Low on a personality facet was defined as a score of ≤ 1SD below the mean of the trait, high as ≥ 1SD above the mean. Significant associations represent results from partial correlation analyses, adjusted for sex, experiment time and habitual smoking and are indicated with asterisks (*). All cortisol values are in nmol/l. CM = cortisol morning.

All correlations were controlled for smoking, experiment time and sex (see Bouma et al., 2009 for more details). Basal cortisol was significantly related to four out of six personality facets. Adolescents with higher levels of basal cortisol were higher on both impulsivity and vulnerability but lower on assertiveness and self-discipline (Figure 1). No significant associations were found between basal cortisol and either angry/hostility and excitement seeking. CAR, anticipation, reactivity, recovery and STAUCg were not related to any of the personality facets.

Additionally, we tested whether the results hold when CAR was operationalized as a standardized residual and reactivity and recovery were operationalized as change scores. Partial correlations showed that associations with personality traits were the same as for the original operationalizations, that is, none of the associations with temperament traits was significant.

Table 3. *Partial correlation analyses.*

	Vulnera-bility	Impulsive-ness	Angry Hostility	Assertive-ness	Excitement Seeking	Self Discipline
Basal	**.206***	**.174***	.072	**-.119***	.022	**-.226***
CAR	.023	-.012	.034	.006	-.032	-.109
Anticipation	-.002	-.036	.060	.029	-.050	.018
Reactivity	.035	.054	-.044	-.072	.019	.019
Recovery	.022	.074	.055	.004	-.042	-.070
STAUCg	.015	.016	-.011	-.035	.004	.015

Note. Results reflect partial correlations. Smoking, experiment time and sex were included in all analyses as covariates. **Bold** = significant association at $p < .05$. CAR = cortisol awakening response; STAUCg = stress task area under the curve with respect to the ground.

DISCUSSION

The aim of this study was to examine whether and how various aspects of HPA-axis functioning were associated with facets of personality in a large population sample of adolescents. In line with our hypothesis, our results showed that individual differences in basal cortisol levels were related to individual differences in certain personality facets. Adolescents with high basal cortisol levels were higher on impulsivity and vulnerability, and lower on assertiveness and self-discipline. We found no association with the other cortisol measures, nor did we find an associations between HPA-axis functioning and either angry/hostility or excitement-seeking.

Basal cortisol and personality

As expected, we found that basal cortisol levels were related to several facets of personality, probably because of the more trait-like nature of basal cortisol (Bartels et al., 2003; Federenko et al., 2004; Hellhammer et al., 2007; Wüst et al., 2000). Moreover, the strength of the effects is

probably an underestimation of the real associations, since previous research has suggested that basal cortisol levels fluctuate across days due to situational factors like waking time and subjective stress load for the prior and upcoming day (Hellhammer et al., 2007). In our sample, situational variability between participants was relatively small since morning cortisol measures were collected at the same day as the behavioural experiments in 95% of the adolescents, resulting in large similarity between adolescents with respect to the upcoming day. Additionally, also the relatively low internal consistency of the personality facets is likely to suppress the correlations between personality and HPA-axis functioning, resulting in an even stronger underestimation of the associations.

The theoretical basis for the association between basal cortisol levels and personality seems to be substantial, but what does the direction of the effects mean? From a meta-analysis on HPA-axis functioning and depression in children, we know that higher basal cortisol levels are related to higher levels of depression (Lopez-Duran, Kovacs, & George, 2009). We found that higher basal cortisol levels were associated with higher levels of two facets of neuroticism: impulsivity and vulnerability. Taking into account the strong relation between neuroticism and depression, our results seem reasonable. In addition, previous research has emphasized the adaptive value of self-discipline (e.g., Oldehinkel, Hartman, de Winter, Veenstra, & Ormel, 2004). We found that high basal cortisol levels were associated with low levels of self-discipline. Taken together the findings on impulsivity, vulnerability and self-discipline, it seems that high basal cortisol is an indication of dysfunctioning of the HPA-axis, and subsequently for vulnerability to psychopathology

From our findings it is not clear how to interpret the negative association between basal cortisol and assertiveness. The literature on extraversion, a concept closely related to assertiveness, has provided evidence for an association with externalizing behaviour problems (John, Caspi, Robins, Moffitt, & Stouthamer-Loeber, 1994). However, not only low, but also high levels of basal cortisol have been related to externalizing behaviour problems (Ryan, 1998). From a person-centered approach these contradicting results might not be surprising given the finding that high scores on extraversion are mostly related to behavioural problems in the presence of other characteristics, like low self-discipline (e.g., Mervielde, De Clercq, de Fruyt, & Van Leeuwen, 2005). More research is needed to improve our understanding of different mechanisms underlying the associations with respectively low and high basal cortisol.

Basal cortisol was related to most, but not all of the personality facets in our study. For example, the neuroticism facet angry/hostility was not related to cortisol, in contrast to the neuroticism facets impulsivity and vulnerability which were positively associated with basal cortisol. This finding seems in line with literature on personality facets suggesting that facets within the same domain may vary in the extent to which they are related to psychopathology. For example, although extraversion has previously been related to externalizing problem behaviours (John et al., 1994), the extraversion facet excitement seeking has a lower threshold for maladaptivity than warmth, which is also a facet of extraversion as measured with the

NEO-PI (Widiger & Trull, 1992). This suggests that it is important to study facets instead of, or in addition to, the broader personality traits like the big three or big five. Unfortunately, not all facets of all NEO-PI personality traits were assessed in our sample due to constraints on the total number of items in the multidisciplinary TRAILS study. Nonetheless, by including the current facets we could differentiate between facets of those personality traits that have been consistently found to be related to psychopathology (Kotov et al., 2010).

CAR and stress-induced cortisol and personality

The absence of associations between personality and both CAR and stress-induced cortisol seem to be in line with the literature. Consistent with our study, the few studies previously performed on CAR generally found no evidence for an association (e.g., Chan et al., 2007). Portella and colleagues (2005) reported a positive association, but they selected participants who scored extremely high or low on neuroticism which makes comparison with other studies difficult (Portella et al., 2005). Previous studies investigating the association between reactivity to a laboratory social stress task and several personality traits yielded inconsistent results. For example, high levels of neuroticism have been related both to elevated (e.g., Habra et al., 2003) and blunted (Phillips et al., 2005) cortisol responses. As was pointed out in the introduction, CAR, anticipation, activation and recovery may not be as trait-like as basal cortisol and therefore be not as strongly related to personality traits. As is evident from Table 2, it is unlikely that the main reason that these measures are not linked with personality is that all of them (except anticipation) were operationalized as change scores or as standardized residuals. None of the single-time cortisol measures were correlated with our personality facets, except CM2, which was positively related to Vulnerability and negatively to Self-discipline. This is probably due to the relatively high correlation with cortisol concentrations at awakening (CM1, $r = .51$). For example, more vulnerable individuals wake up with higher cortisol concentrations, but show similar cortisol awakening responses (CAR), resulting in similarly higher levels of CM2. No associations were found with either Impulsivity or Excitement seeking and none of the single cortisol samples collected during the social stress task was related to (one or more) personality traits. This seems to bolster the argument that basal HPA-axis activity, but not reactivity, is inherently relevant to personality.

Sex differences in personality and HPA-axis functioning, as well as in the association between personality and HPA-axis functioning were explored. With regard to personality, our findings are well in line with previous studies (for a meta analysis see: (Roberts, Walton, & Viechtbauer, 2006). Girls were higher on vulnerability and impulsivity, both facets of emotional instability, whereas boys tended to be higher on assertiveness and excitement seeking, both facets of extraversion. Concerning HPA-axis functioning, sex differences in cortisol reactivity to stress have been found to be modest, reporting slightly stronger increases in boys (e.g., Kudielka & Kirschbaum, 2005 for a review). The results of the current study were in line with these findings. Higher cortisol levels were found in boys both prior to the stress task and

during the stress task, as well as a larger reactivity. Girls showed stronger recovery after the task. However, given that HPA-axis functioning has been suggested to be very sensitive for differences in task design (Burt & Obradović, 2012), caution is needed when comparing our findings with previous literature. Finally, we investigated sex differences regarding the bivariate correlations between personality and HPA-axis functioning. No consistent sex differences were found, and therefore our main analyses were performed for boys and girls together. Previous studies have usually not reported on sex differences. This may be a result of the small samples and limited power. It may also be that sex differences were not reported because associations were simply the same for boys and girls, which would be in line with our findings. Future studies using adequate sample sizes should investigate and report on this.

Compared to other studies in this field, our sample was very large. Next to the advantage of higher power, is the advantage of smaller influence of outliers. Furthermore, our study is the first investigating the association between personality facets and various indices of HPA-axis activity representing different physiological functions. The direct comparison of associations with various aspects of HPA-axis functioning is novel to the literature, as well as the inclusion of anticipation and recovery. We have attempted to maximize similarity between the operationalization of our cortisol indexes and operationalizations in the literature (e.g., difference scores for CAR and standardized residuals for reactivity). Interestingly, when using other operationalizations (i.e., standardized residuals for CAR, change scores for reactivity and recovery) our findings remained the same. Therefore, it seems plausible that other operationalizations than the ones used in the current study will result in similar findings than the ones we found. It should be noted however, that the current sample was initially selected with a slightly elevated risk (e.g., for familial psychopathology), to gain statistical power in the 'high-risk range' and subsequently, to get more information on a relatively interesting subgroup of adolescents. Consequently, although this 'focus sample' still represented the whole range of problems seen in a normal population (Oldehinkel & Bouma, 2011), replication in a fully representative cohort-sample is needed.

In conclusion, our study is one of the first providing evidence that basal cortisol, is related to facets of the personality traits neuroticism, extraversion and conscientiousness. In line with previous studies, stress-induced cortisol was not consistently related to personality. These findings suggest that, possibly due to its more trait-like nature, basal cortisol seems to be is most informative when investigating more trait-like characteristics such as personality facets.

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4

Stressful events and temperament change during early and middle adolescence

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ABSTRACT

This project investigates how stressful events are related to deviations from normative temperament development during adolescence. Temperament traits were assessed at age 11 and 16. Life event data were captured using an interview (n = 1197). Normative changes were found in all traits. A linear trend was found between the experience of stressful events and temperament development. Adolescents exposed to stressful events showed smaller decreases in fear and shyness, stronger decreases in effortful control and affiliation and smaller increases in high intensity pleasure. Exposure to stressful events was related to increases in frustration instead of decreases. Our results show that while normative development is mostly in the direction of maturation, adolescents who experienced stressful events showed less maturation of their temperament.

INTRODUCTION

How are stressful events related to changes in temperament traits? Previous studies have suggested that exposure to major stress can be related to a heightened vulnerability to the development of mental disorders later in life (Rutter, 2006). It is unknown, however, whether stressful experiences can result in more fundamental changes. This study has the aim to investigate associations between stressful events and temperament from age 11 to 16.

Adolescence

Adolescence is a period characterized by major biological, psychological and social changes as well as by intense interactions with the environment. These changes, in combination with the increased brain plasticity typical for adolescence, raises the question if, and to what extent, adolescence can be seen as a window of vulnerability and opportunity (Spear, 2000). If adolescence is a vulnerable period, stress during this period should have enduring consequences for the adolescent's development (Lupien, McEwen, Gunnar, & Heim, 2009). However, literature on the impact of stressful events during adolescence is scarce and it is unknown how stress is related to the development of temperament during this period. Therefore we will investigate whether adolescents exposed to stressful events show changes in temperament deviating from normative adolescent temperament change.

Temperament

Until recently, temperament has been conceptualized as a predominantly biologically based precursor of later personality. Personality was generally seen as less constitutional, but broader and more differentiated than temperament (Shiner, 2006). Over the last years, the distinction between temperament and personality has been debated (e.g., Clark, 2005). An increasing emphasis on the connection between personality and temperament has emerged with the suggestion that all of the major features of personality (i.e. stability; heritability) equally characterize temperament traits (Costa & McCrae, 2001). From this perspective, personality and temperament are largely equivalent and the terms may even be used interchangeably (Caspi & Shiner, 2006; Klein, Kotov, & Buffered, 2011). Consequently, they can both be used when studying differences in traits between people. This study uses the Revised Early Adolescent Temperament Questionnaire (EATQ-R), a questionnaire developed specifically for children in the early adolescent years. The questionnaire measures six traits: fear, frustration, affiliation, high intensity pleasure, shyness and effortful control (Oldehinkel, Hartman, de Winter, Veenstra, & Ormel, 2004; Putnam, Ellis, & Rothbart, 2001). The EATQ-R seems compatible with measures like the Big Three or Big Five. High positive correlations have been reported between respectively fear and frustration and emotional instability and between high intensity pleasure and extraversion (Muris, Meesters, & Blijlevens, 2007). Rothbart and colleagues have suggested similar associations, as well as relations between effortful control and conscientiousness (Rothbart, Ahadi, & Evans, 2000).

Temperament change

The notion of traits (both temperament and personality) as characterizing differences between people implies a certain level of stability. Traits measured in childhood are suggested to be predictive of traits measured during adolescence and adulthood. Despite this assumed stability, extensive literature on stability and change has suggested that traits are not developmentally static (e.g., Caspi, Roberts, & Shiner, 2005; De Fruyt et al., 2006). Instead, traits have generally been approached as more or less dynamic dimensions of interindividual differences (Costa Jr, Herbst, McCrae, & Siegler, 2000; Rothbart et al., 2000).

Trait consistency at the population level can be studied by looking at mean-level changes (reflecting whether groups of people increase or decrease on a trait over time) as well as by looking at rank-order consistency (reflecting changes in relative placement of individuals in a group; Roberts & DelVecchio, 2000). The current study will address both.

Changes in traits can have different sources. Changes may result from intrinsic maturational factors or from environmental factors, such as a pressure to behave according to social roles, but also idiosyncratic life events. Extensive literature has suggested developmental changes in traits; often in the direction of maturation (Klimstra, Hale III, Raaijmakers, Branje, & Meeus, 2009; McCrae et al., 2002; Roberts, Wood, & Smith, 2005; Robins, Noftle, Trzesniewski, & Roberts, 2005). For example, during adolescence mean-levels of emotional instability have often been found to decrease (but not always in girls, McCrae et al., 2002), whereas levels of extraversion have often found to increase (but not always in boys, Branje, van Lieshout, & Gerris, 2007; for a review see Caspi et al., 2005). With regard to conscientiousness, developmental changes towards maturation have mostly been reported after age 18 (Caspi et al., 2005). Between age 11 and 18 most studies have found either stability or decrease, suggesting that change towards maturation is not normative in this age group (e.g., Allik, Laidra, Realo, & Pullmann, 2004; Pullmann, Raudsepp, & Allik, 2006).

Rank-order stability has been studied by Roberts and DelVecchio (2007) in a large quantitative review. Analyses of more than 150 longitudinal studies suggested that trait consistency increased from .31 in childhood to .54 during college years. This indicates a decrease in the amount in which people change in ordinal position over time.

The current study is the first using the EATQ-R to look at normative, or mean-level changes. In line with previous findings decreases are hypothesized with regard to fear and frustration (both related to emotional instability) and increases in high intensity pleasure (related to extraversion). Regarding effortful control (related to conscientiousness) stability or a small decrease is hypothesized. Changes in shyness and affiliation as well as gender differences are explored. With regard to rank-order consistency, we hypothesize moderate stability coefficients.

Stressful events and temperament change

Temperament change does not only occur at the group-level, but can also differ between individuals (Branje et al., 2007). These interindividual differences in temperament change are most likely to result from environmental factors, like life events. Evidence for stressful events being directly related to changes in traits is scarce. Some support for stress affecting changes in traits at least for some years, can be found in research with adults. Reporting an extremely adverse event has recently found to be related to increases traits, e.g., emotional instability, over an eight-year period (Löckenhoff, Terracciano, Patriciu, Eaton, & Costa, 2009). Also other studies have suggested that stressful events are associated with changes in traits related to emotional stability (Costa Jr et al., 2000; Mroczek & Spiro, 2003; Vaidya, Gray, Haig, & Watson, 2002). For example, Costa and colleagues reported that fired individuals showed increases in different facets of emotional stability (anxiety, depression and vulnerability). Increases in the depression facet were found in men after divorce. In a prospective and genetic study with mono-and dizygotic twins, reciprocal causation was suggested to explain the association between life events and emotional stability (Middeldorp, Cath, Beem, Willemsen, & Boomsma, 2008). Except for the study by Costa and colleagues (2000) in none of these studies gender differences were found or reported.

The current project will extend these studies by investigating associations between stressful events and changes in EATQ-traits during adolescence. As traits are supposed to be more stable during later life than during adolescence, adolescents may be more sensitive to stress-related change than adults. However, the direction of the effects is expected to be comparable for adolescents and adults. To improve generalization we will focus on events that have been studied previously. For events that are adult specific we have studied comparable events that do occur during adolescents (e.g., marital problems or divorce may be comparable with the stressful event of losing a friend by a fight or argument during adolescence).

We hypothesize that adolescents exposed to stressful events show increases in fear and frustration instead of the normative decreases expected to be found. These hypotheses are consistent with findings of the adult studies mentioned before. Associations with high intensity pleasure, effortful control, shyness and affiliation are more exploratory. As there are no clear indications of gender differences, differences between boys and girls in the associations between stressful events and changes in traits are explored.

Associations between stressful events and temperament are likely to differ dependent on the number of events experienced by the adolescent. Studies on stress and children's adjustment have suggested that whereas the magnitude of the effect of a single risk factor is often relatively small, multiple stressful events may accumulate to interfere with adjustment (Forehand, Biggar, & Kotchick, 1998). Therefore, the current project will investigate the relation between cumulative stress and adolescent temperament change.

Finally, when studying the stressful events the question raises whether all adolescents are equally vulnerable to the influence of stressful events. We will study this by taking into account baseline levels of traits.

Summary

In sum, this project will investigate the role of stressful events on temperament change during early and middle adolescence. It will be the first study reporting on normative changes in EATQ-R traits during this age period. Changes towards maturation are expected in fear and frustration. Increases are hypothesized with regard to high intensity pleasure and stability or decrease is expected in effortful control. With regard to rank-order consistency moderate stability coefficients are expected. Then, we will investigate whether adolescents exposed to stressful events show changes in temperament deviating from normative adolescent temperament change. It is hypothesized that stressful events are related to increases in fear and frustration.

METHODS

Sample

The TRacking Adolescents´ Individual Lives Survey (TRAILS). TRAILS is a large prospective cohort study of Dutch adolescents, who are followed bi- or triennially from 11 to at least 25 years of age. The present study involves data from the first and third assessment wave. The waves ran from, respectively, March 2001 to July 2002 and September 2005 to December 2007. A detailed description of the sample selection, procedures and methods can be found in (de Winter et al., 2005). At the start of the project the target sample involved all 10- to 11-year-old children living in the north of the Netherlands, in both cities and rural areas. Selected municipalities were requested to give out names and addresses of all children of the target group (3483 names). At the same time, schools were asked to participate. School participation was a prerequisite for children and parents to be asked to enrol in the study. Of the 135 primary schools in the area 90.4% of the schools, accommodating 90.3% of the children agreed to participate. Then both parents and children were asked for agreement to participate.

At wave 1, 2230 pre-adolescents (50.8% girls) enrolled in the study (response rate 76.0%) of whom 1816 (response rate 81.4%, 45.3% girls) participated in wave 3. At wave 1 the mean age of the adolescents enrolled in the study was 11.09 (SD = 0.56). At wave 3 the mean age was 16.13 (SD = 0.59). Two prerequisites to be included in the current study were that the parents had filled out the temperament questionnaires of age 11 and 16 *and* that the adolescents were interviewed with regard to events. This resulted in a total number of 1197 adolescents participating in the current study.

Our sample can be regarded as representative for the adolescent population from the north of the Netherlands. No differences were found between responders and non-responders with respect to teacher ratings of problem behaviours and on the associations between socio-demographic variables and mental health indicators. We examined whether individuals who were interviewed with regard to events differed from those who were not interviewed on the temperament scales at age 11. To facilitate comparisons partial-eta-squared measures

of effect (η^2) were computed. The effect sizes for being interviewed were all smaller than .01, which can be interpreted as negligible effects (Cohen, 1988). Only for affiliation and effortful control the differences were statistically significant at $p < .05$. Thus for these two scales there was evidence that attrition was non-random (higher in children low on effortful control and low on affiliation). However, effect sizes were so small (respectively, partial $\eta^2 = .002$ and .003), that our results do not seem to be seriously biased.

Procedures

For the first measurement wave of the TRAILS project, well-trained interviewers visited one of the parents or guardians (preferably the mother, 95.6%) at their homes. Parents were asked to fill out a written questionnaire, including questions about the child's temperament. Children and teachers were asked to fill out questionnaires at school. When adolescents were 16 years old, a similar procedure was used. In addition, children were interviewed at a central facility in the child's home area by well-trained interviewers in order to collect life event data.

Measures

Temperament. Child temperament was assessed both at age 11 and at age 16 by means of the short form of the parent version of the Early Adolescent Temperament Questionnaire-Revised (EATQ-R, Hartman, 2000; Putnam et al., 2001). We used the parent version as, in our sample, the factor structure of this version was superior to that of the child version (Oldehinkel et al., 2004). Because the scales as proposed by Rothbart and co-workers had not been verified empirically in large population samples, principal component analysis was used to investigate the extent to which the original scales reflected the structure of the EATQ-items in the TRAILS sample. This led to some minor alterations of the original scales (Oldehinkel et al., 2004). The following six scales were distinguished: fear (negative affect related to anticipated pain or distress, five items, Cronbach's $\alpha = .63$), frustration (negative affect related to interruption of ongoing tasks or goal blocking, five items, $\alpha = .74$), shyness (slow or inhibited approach and/or discomfort in social situations, four items, $\alpha = .84$), effortful control (capacity to control attention, activation and inhibition, 11 items, $\alpha = .86$), affiliation (desire for, and pleasure in, warmth and closeness with others, six items, $\alpha = .66$) and high intensity pleasure (pleasure or enjoyment related to high stimulus intensity or novelty, six items, $\alpha = .77$). Answers were rated on a 5-point Likert-type scale (1 = "almost always untrue" to 5 = "almost always true"). Higher values indicated a higher presence of the temperamental trait concerned. Missing items were imputed by means of Corrected Item Mean imputation (CIM; Huisman, 2000). Test-retest stability of the EATQ-R scales has been found to be moderate to good, ranging from .69 for high intensity pleasure to .85 for frustration (Muris & Meesters, 2009).

Event History Calendar. Stressful events were captured at age 16 using the Event History Calendar (EHC), a data collection method for obtaining retrospective data about life events and activities (Caspi et al., 1996). For the present study the calendar as developed by Caspi and

co-workers (1996) was adapted into an interview on several life domains that lasted about 45 minutes. Participants were asked about events that occurred since the first assessment (i.e., between ages 11 and 16). Detailed and accurate data about the events could be collected by proceeding serially from one life domain to another and using a month-by-month horizontal timeline. For example, with regard to school, adolescents were asked respectively about the dates of changing school, changing class, repeating class, as well as about their educational levels for the subsequent years.

Test-retest reliability has generally been found to be reasonable to good (respectively, 72-87% in a sample of young adults; Freedman, Thornton, Camburn, Alwin, & De Young, 1988; > 90% in a sample of adolescents; Caspi et al., 1996). Construct validity of the Event History Calendar was investigated in a comparative study by Belli and colleagues (Belli, Shay, & Stafford, 2001). In this study reasonable correlation coefficients were found between a written questionnaire and the EHC (ranging from .63 to .79).

Ten events were singled out for the current project based on previous studies on stressful events (e.g., McMahon, Grant, Compas, Thurm, & Ey, 2003). All events are presumed to be relevant stressful events during adolescence. Events selected were: house move, parental divorce, death of a direct family member (i.e. mother, father or sibling), illness of a direct family member (severe, physical illness;), death of a good friend, being expelled from school, running away from home, repeating a class, being thrown out of the parental home, end of a friendship caused by a fight or an argument. For this study, an event variable was constructed, indicating the number of stressful events the adolescence experienced.

Strategy of analysis

Normative temperament change and rank-order stability. First it was tested whether normative changes could be found in the six temperament traits. Univariate ANOVA's in SPSS were done using the scores on the six temperament traits (as measured at age 11 and 16) as within subject variables. Separate analyses were used as the temperament traits correlated only low to moderate (max. -.37, $p < .001$ for the correlation between frustration and effortful control at age 11). To test whether boys and girls differed in temperament change, gender was included as a between subject variable. Rank-order stability was investigated using test-retest correlations.

Stressful events and temperament change. Analysis of the associations between stressful events and temperament change was done using regression analyses of the event variable and Reliable Change scores (RCscores; Jacobson & Truax, 1991). RC scores are difference scores which take unreliability of measurement explicitly into account ($RCscore = (X_2 - X_1)/S_{diff}$, in which X_1 and X_2 are the scores on the EATQ-scales at age 11 and 16 and S_{diff} is the standard error of the difference between scores at age 11 and 16; Christensen & Mendoza, 1986), thereby making separation possible between true changes in temperament and changes due to measurement error. This explicit correction for measurement error makes RCscores preferable to more common techniques. In all associations possible gender differences were taken into

account. To test the possible moderating effect of baseline temperament the sample was split into two, those who were initially higher on a trait and those who were initially lower on a trait.

RESULTS

Descriptive statistics

Descriptive statistics for temperament at age 11 and 16 are reported in Table 1. Stressful events differed in the frequency they were experienced by adolescents: house move $n = 275$, parental divorce $n = 89$, death of a direct family member $n = 19$, illness of a direct family member $n = 126$, death of a good friend $n = 22$, being expelled from school $n = 50$, running away from home $n = 52$, repeating a class $n = 184$, being thrown out of the parental home $n = 20$, end of a friendship caused by a fight or an argument $n = 128$. The total number of events adolescents were exposed to ranged from zero to five events. Three, four and five events were combined to retain an acceptable power. 46.6% of the adolescents were exposed to zero events; 34.1% to one event; 12.6% to two events and 6,7% to three or more events. Correlations between stressful events were all low, (max. .174, $p < .001$ for the correlation between illness of a family member and death of a family member), as well as were the correlations between stressful events and temperament traits (max. -.166, $p < .001$ for the correlation between being expelled from school and effortful control at age 16).

Table 1. *Descriptive Statistics of EATQ traits at age 11 and 16 and Univariate Repeated Measures Analyses (N = 1197).*

	Age 11		Age 16	
	Boys	Girls	Boys	Girls
Trait		*M (SD)*	*M (SD)*	*M (SD)*
Fear[abc]	2.33 (0.69)	2.47 (0.73)	1.82 (0.65)	2.05 (0.65)
Frustration[abc]	2.83 (0.67)	2.71 (0.64)	2.66 (0.72)	2.73 (0.69)
Shyness[ab]	2.39 (0.85)	2.60 (0.87)	2.23 (0.90)	2.42 (0.91)
Affiliation[abc]	3.76(0.58)	4.00 (0.51)	3.49 (0.69)	3.86 (0.55)
High intensity pleasure [abc]	3.04(0.95)	3.21 (0.93)	3.55 (0.86)	3.45 (0.84)
Effortful control[ab]	3.13(0.71)	3.40 (0.65)	3.04 (0.65)	3.36 (0.63)

Note. [a]Boys and girls differ significantly at age 11 at $p < .01$. [b]Changes in trait (time effect from age 11 to 16) significant at $p < .001$. [c]Time x gender effect significant at $p < .05$.

Normative temperament changes and rank-order stability

Results of the repeated-measures are included in Table 1. Univariate analyses showed decreases in all traits except in high intensity pleasure, for which scores increased. Respectively for fear $F(1195,1) = 524.87$, partial $\eta^2 = .305$, for frustration $F(1195,1) = 14.08$, partial $\eta^2 = .013$, for shyness $F(1195,1) = 53.98$, partial $\eta^2 = .043$, for affiliation $F(1195,1) = 135.76$, partial $\eta^2 = .102$, for effortful control $F(1195,1) = 14.14$, partial $\eta^2 = .012$, and for high intensity pleasure

F (1195,1) = 61.31, partial η^2 = .049. All effects were significant at p < .001. Effect size of the changes differed largely between the various traits, with only the decreases in fear being highly substantial (Cohen, 1988). Gender differences were found with regard to fear F (1195,1) = 4.35, p < .05, frustration F (1195,1) = 21.60, p < .001, affiliation F (1195,1) = 15.65, p < .001, and high intensity pleasure F (1195,1) = 4.25, p < .05, with boys showing stronger changes then girls except on high intensity pleasure. With respect to frustration girls showed a weak increase, whereas boys decreased strongly on the trait. The effect sizes of gender differences in temperament change were small (< .018).

Test-retest correlations showed moderate rank-order stability in all traits. Stability coefficients ranged from .48 for fear to .56 for shyness.

Stressful events and temperament change

The experience of stressful events predicted changes in all temperament traits. Being exposed to more stressful events was related to *deviation from the normative changes* shown by adolescents not exposed to stressful events. Associations between the number of stressful events and temperament change are depicted in Figure 1. Overall, linear effects of the number of stressful events experienced on temperament change were found. The more stressful events an adolescent experienced the larger his or her changes in temperament deviated from normative temperament change. Adolescents exposed to stressful events showed smaller decreases in fear and shyness, than adolescents who were not exposed to these events (B = .410, SE = .092, β = .370, p < .001, and B = .069, SE = .032, β = .062, p = .031, respectively). So, the more events the smaller the decreases found between age 11 and 16. Being exposed to more stressful events was related to stronger decreases in effortful control and affiliation (B = -.108, SE = .032, β = -.097, p = .001, and B = -.117, SE = .032, β = -.105, p < .001, respectively). Regarding high intensity pleasure, stressful events were related to smaller increases (B = -.069, SE = .032, β = -.062, p = .032). Finally, adolescents exposed to stressful events showed smaller decreases or even increases in frustration instead of the decrease found in adolescents not exposed to stressful events (B = .142, SE = .032, β = .128, p < .001).

Stressful events*gender interaction terms were included to investigate gender differences in the association between stressful events and temperament change. None of the interaction terms we included were significant. Similar analyses were done with baseline temperament. Only for fear the association between stressful events and temperament changes was moderated by baseline temperament levels. Adolescents high in fear at baseline showed on average larger decreases than adolescents initially low B = -.739, SE = .044, β = -.527, p < .001), and, most important, were less sensitive to the influence of stressful events B=-.100, SE = .036, β = -.238, p = .006). For the other traits interaction terms were not significant.

Overall, being exposed to stressful events was related to all temperament traits. The more stressful events, the stronger the deviations from normative temperament change. However, some traits were more affected by stressful events then others. Association between stressful

events and temperament change were the same for boys and girls. Baseline temperament levels only moderated the association between stressful events and fear. For this trait more variance was explained in the model than for the other traits (fear R^2 = .368, frustration R^2 = .016, shyness, R^2 = .004, affiliation R^2 = .011, effortful control R^2 = .009, high intensity pleasure, R^2 = .004).

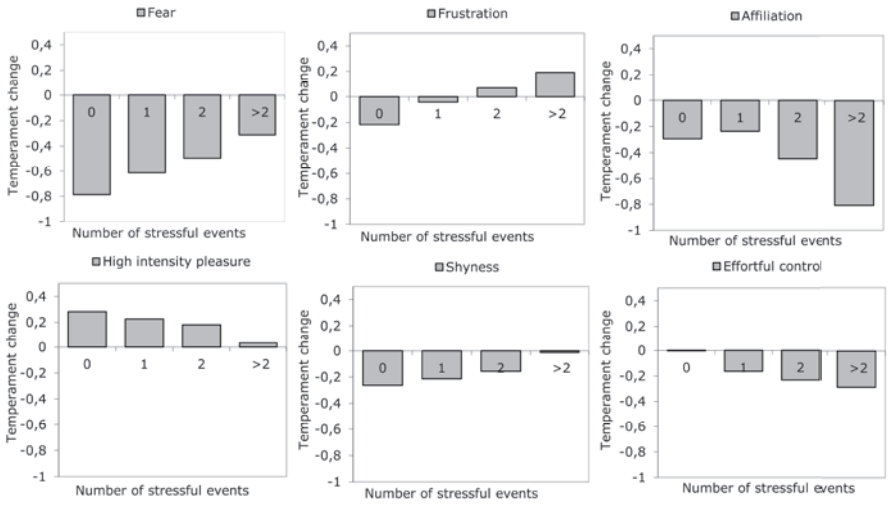

Figure 1. Associations between number of stressful events and temperament change. The y-axis represents *change* between age 11 and 16 as measured with the RC scores. Scores below the zero-line represent *decreases*, whereas scores above the zero-line represent *increases* in the trait.

DISCUSSION

In the current study we examined the influence of stressful events on changes in temperament during adolescence. Our results showed that adolescents exposed to stressful events showed changes in temperament traits which slightly deviated from normative adolescent temperament change.

Normative temperament change and rank-order stability

To examine how temperament change shown by adolescents exposed to stressful events deviated from normative temperament change, we first investigated normative temperament change. Adolescents showed significant decreases in all temperament traits except in high intensity pleasure, for which scores increased significantly. Although this study was the first to look at normative changes in EATQ-R traits, our findings were comparable with changes in traits previously reported based on the Big Five. In line with the idea of development towards maturation, traits related to emotional instability (fear and frustration) decreased over time, whereas high intensity pleasure (related to extraversion) increased. As has been previously

suggested with regard to conscientiousness, effortful control did not show changes in the direction of maturation. It might be that effortful control matures late compared to other traits or that it even shows a temporary relapse effect between age 11 and 16 (Allik et al., 2004). Affiliation showed a reasonable decrease. This trait is less comparable with Big Five traits, so normative changes have been largely unstudied. The decreases found suggest that, like with effortful control, changes were not (yet) in the direction of maturation between age 11 and 16. Nonetheless, generalization of this finding needs some caution as it is based on the parent-version of the EATQ-R. It might be that parents mainly referred to decreased affiliation in the adolescent-parent relationship and were insufficiently informed about affiliation regarding adolescent-friend relationships.

In the current study all temperament traits were found to change during adolescence. This seems to contradict previous findings, often reporting changes in some, but not all, traits. The trait most consistently found to be sensitive to change has been emotional instability (Caspi et al., 2005). Largest effect sizes were in our indeed found for fear, a traits closely related to emotional instability. The EATQ-R approaches facets of emotional stability as separate scales (i.e., fear, frustration), whereas (Big Five) traits less often found to change in previous studies (like openness to experience) were not studied with the EATQ-R. This might explain that we found more traits changing than previous studies. In addition, the EATQ-R traits less related to emotional instability showed significant, but very small changes that would probably not be significant in studies with fewer participants. Small sample sizes are indeed one of the limitations which may explain the lack of consistent findings (Klimstra et al., 2009). As we studied normative development in a large population cohort this limitation does not apply to our results. The other limitations such as high attrition rates and specific sample characteristics (Klimstra et al., 2009) were not applicable to our study either. Thus, normative development in our sample can be regarded as reliable and largely generalizable.

With regard to the gender differences we explored, all differences were small but with boys changing stronger than girls on most traits. This is probably due to the developmental difference between the ages 11 and 16 which might be larger for boys than for girls as a result of their later transition into adolescence and later personality maturation (Klimstra et al., 2009).

Analyses of rank-order stability showed moderate test-retest coefficients for all traits. These results are in line with the findings of Roberts and DelVecchio (2000), described in their meta-analysis.

Stressful events and temperament change

Subsequently, we examined our main question: Do adolescents exposed to stressful events show changes in temperament deviating from normative adolescent temperament change? Confirming our hypothesis, the results showed that stressful events were indeed related to disruption of the adolescents' development towards a mature temperament. Adolescents exposed to stressful events showed increases in fear instead of the expected decreases. With

regard to frustration, adolescents who were exposed to several stressful events even showed the reverse of maturation (increases instead of decreases). Effortful control and affiliation showed changes between age 11 and 16 that seemed the reverse of maturation, when exposed to stressful events deviation from maturation seemed even stronger. Also for the other traits associations were found between stressful events and deviations from normative change. Gender differences were explored but non-significant in all associations. This seems in line with previous studies were gender differences were not found or not reported.

To study initial vulnerability to the influence of stress, we looked whether baseline levels of traits were related to the association between stressful events and temperament change. Only for fear this association was significant, but associations were not in the expected direction. Our finding that adolescents with higher baseline levels seemed less sensitive to the influence of stress on changes in fear, can probably be explained by a statistical ceiling effect. Future work may prevent this by using another measure for initial vulnerability.

The strength of the effects was small for all traits, although fear was more sensitive to the influence of stressful events than other traits. This would be in line with the suggestion presented by Mroczek and Spiro (2003) that neuroticism has greater plasticity than extraversion, or that rate of change in extraversion is influenced more by other types of variables than by life events. It might for example be that high intensity pleasure (related to extraversion), which was only slightly related to stressful events, is more sensitive to positive than to negative events.

So, although all associations were significant, the strengths of the effects were often small or very small. Nonetheless it seems clear that stressful events are negatively associated with normative temperament change. Moreover, our results consistently showed linear trends; the more stressful events adolescents experienced, the stronger their temperament change deviated from normative development. This may suggest that our study only reveals the top of the iceberg. Some adolescents may be exposed to more than the maximum of five we found in our study as we did only look at ten events. And these events may also be more stressful than some of the events we measured. Those adolescents, exposed to more or more severe stress, may show much stronger deviations from normative temperament than the deviations reported in our study. We used a population-based sample in which (several) stressful events did not occur very often. This sample had the advantage of being indicative for the Dutch adolescent population, but the limitation that it was difficult to study the objective impact of stress in depth. With a focus sample of adolescents exposed to more or more severe stressful events this might be easier. In a population sample like ours, stronger associations can probably be found when a measure of the impactfulness or contextual severity of the events is used. House moves are likely to cause less stress than parental divorce, but depending on the context in some cases the opposite might be true as well. A stress instrument including contextual stress scores can distinguish between mild and severe stressors.

In addition, the subjective experience of the impact of the stressful events was not measured. With data on subjective stress experience it might be possible to measure

differences in (initial) vulnerability in more detail than we did. Adolescents who are high on a vulnerability dimension may score stressful events as more severe than adolescents who are lower on this dimension. As a result, changes in traits may be elaborated by the vulnerability dimension-correlated subjective experiences that they create.

In contrast to the severe stress that is likely to be related to temperament change, mild stress is relatively common during the adolescent years. Our results have shown that these mild stressors should not be ignored when studying stress and temperament change. Even mild stress is related to deviations in temperament development, especially when multiple events were experienced. For example, based on our findings it seems that if parents have jobs that require frequent moving due to internal or international secondments, this might be harmful for adolescents' personality maturation. We do not yet know how lasting the deviations from normative temperament change are. It seems likely that the impact of stressful events in itself is not irreversible, but that stressful events set in motion person-environment transactions. For example, being expelled from school may lead to increases in frustration, and high levels of frustration may in turn lead to an increased risk for experiencing this type of events again. In this way an event may reflect the characteristics of an individual that selected him or her into this situation. So stressful events, that are a consequence of the adolescents' own action (person-dependent events), are likely to reinforce and sustain the adolescents previous existing traits, whereas person-independent events, like the death of a friend, are unlikely to set in motion such transactions (Caspi & Shiner, 2008; Kendler, Gardner, & Prescott, 2003; Ormel & Wohlfarth, 1991). In our study, we could not find evidence for this difference as all our events showed low correlations with temperament, both the once likely to have been a consequence of the adolescence own action and the once more likely to have occurred independent from the adolescents behavior. Future research including more information on the objective context or person-dependency of the event and/or subjective experiences should look at this in more detail.

Conclusion

In conclusion, the present study has added to our knowledge on associations between stressful life-events and changes in temperament traits during early and middle adolescence. Overall, findings revealed that adolescents being exposed to stressful events show changes in traits slightly deviating from normative temperament change. While normative development is mostly in the direction of maturation (e.g., lower fear and frustration), adolescents who experienced stressful events showed less maturation (e.g., lower decreases in fear) or even the reverse of maturation (e.g., increases in frustration) of their temperament.

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5

Normative and adversity-driven changes in hypothalamic-pituitary-adrenal axis functioning

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ABSTRACT

The hypothalamic-pituitary-adrenal (HPA) axis has been suggested as a key mechanism in the transduction of adversity into psychopathology. Little is known about either normative or adversity-driven *changes* in HPA-axis functioning. Using a longitudinal design, we examined changes in five measures of HPA-axis functioning during adolescence (n=141). Adolescents exposed to social defeat (i.e., integrity threatening events) did not show the normative increase to a social stress task found in other adolescents. Instead, they showed a lower cortisol level after the task at age 19 (but not 16) than other adolescents. No changes were found in the other measures. Differential stability was modest to substantial. This study provides unique longitudinal data about normative changes in HPA-axis functioning, and shows that social defeat, but not loss/illness, can affect the body's neuroendocrine response to stress, suggesting that the potential of adverse events to affect HPA-axis functioning is dependent on whether events are integrity threatening.

INTRODUCTION

HPA-axis activity and psychopathology

Major adversity is a well-known risk factor for the development of psychopathology (Beitchman et al., 1992; Kendler, Neale, Kessler, Heath, & Eaves, 1993; Ormel, Oldehinkel, & Brilman, 2001). Over the years scientists have become increasingly interested in the biological mechanisms by which adversity "gets under the skin", and affects vulnerability and mental health (Miller, Chen, & Zhou, 2007). An important underlying mechanism could be adversity driven changes in hypothalamic-pituitary-adrenal (HPA) axis functioning (Herbert, 1997; Susman, 1998). In addition, remarkable little is known on normative changes in HPA-axis functioning. Therefore the current study aims to 1) examine normative mean level changes and differential stability in HPA-axis functioning during adolescence and 2) investigate how adversity is related to deviations from normative changes in HPA-axis functioning.

Normative changes in HPA-axis functioning

Until now, only a few studies examined the developmental course of cortisol, suggesting increases both in basal and in stress-induced HPA-axis functioning from childhood to young adulthood (Trickett, Noll, Susman, Shenk, & Putnam, 2010). Nonetheless, longitudinal data on normative changes in adolescence in stress-induced cortisol activity are lacking. Therefore, the first aim of the current study is to examine normative changes in HPA-axis functioning from middle to late adolescence. Additionally and new to the literature, we will explore rank-order or differential stability, reflecting changes in relative placement of individuals within a group.

Adversity-driven changes in HPA-axis functioning

There is a growing body of evidence that inter-individual variation in exposure to adverse events is related to inter-individual variation in HPA-axis functioning (Heim & Nemeroff, 2001; Kaufman, Plotsky, Nemeroff, & Charney, 2000; Sanchez, 2006). However, findings on these associations are inconsistent. For example, with regard to basal morning cortisol elevated as well as lower levels have been found in individuals exposed to adverse events (Bruce, Fisher, Pears, & Levine, 2009; Cicchetti & Rogosch, 2001; Heim, Ehlert, & Hellhammer, 2000; Miller et al., 2007; Trickett et al., 2010). Also concerning cortisol responses to (laboratory) stress both increased (Heim et al., 2000; Tyrka et al., 2008) and blunted responses were found (Elzinga et al., 2008; MacMillan et al., 2009; Ouellet-Morin et al., 2011).

However, when the nature of the adversity is taken into account, some patterns emerge. Traditionally, a distinction has been made between traumatic and non-traumatic forms of adversity, defining traumatic as experiences that involve "actual or threatened death or serious injury, or a threat to the physical integrity of self or others" (American Psychiatric Association, 2000). In addition to the physical self (e.g., combat), integrity might also be threatened in terms of the social self (e.g., emotional abuse), or both (e.g., sexual abuse). Events that are a

threat to the individuals integrity can elicit intense emotions (Miller et al., 2007) and affect the individuals self-esteem (Jumper, 1995), and may therefore result in more pronounced alterations of the HPA-axis than events that are not integrity threatening. Research findings suggest that the HPA-axis responds with sustained periods of increased cortisol secretion followed by down-regulating cortisol secretion to (integrity threatening) adversity (MacMillan et al., 2009; Miller et al., 2007).

Consistent with this pattern is the "attenuation hypothesis", positing that persistent activation of the HPA-axis eventually downgrades stress reactivity to limit physiological, emotional and behavioural responses to stress (Gunnar & Vazquez, 2001; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Susman, 2006). Although this hypothesis is plausible given the findings mentioned before, only one long-term longitudinal study has actually tested the hypothesis. Trickett and colleagues (2010) found that, whereas increases in basal cortisol levels across development were normative, victims of childhood sexual abuse had somewhat higher basal cortisol levels shortly after disclosure of being abused, but showed smaller increases over time than non-abused children. These findings might suggest cortisol hypo-secretion after a period of heightened secretion (Trickett et al., 2010). It is unknown whether the attenuation hypothesis also holds for stress-induced cortisol activity, in addition to basal activity. Although the need for longitudinal studies has often been mentioned, no studies have been performed on either normative developmental changes or adversity-driven attenuation of stress-induced cortisol activity.

Measures of HPA-axis functioning

The association between adversity and changes in HPA-axis functioning is likely to differ dependent on the measures under study. Almost all studies so far focussed either on basal cortisol or on cortisol increases induced by a social stress task (HPA-axis activation; Koolhaas et al., 2011). However, other measures, such as anticipatory activity and recovery afterwards might also be informative. Whereas *anticipation* reflects unpredictability of the environment, reduced *recovery* afterwards is an indicator of lack of control over the situation. Unpredictability and lack of control are considered the main determinants of stressful situations (Koolhaas et al., 2011). Additionally, two other aspects of HPA-axis functioning can be distinguished: basal activity and the increase after awakening. Whereas basal cortisol levels follow a circadian rhythm in healthy humans and can be seen as a trait component of the HPA-axis (Hellhammer et al., 2007), the cortisol awakening response (CAR) reflects anticipation of demands of the upcoming day (Fries, Dettenborn, & Kirschbaum, 2009). We will include the following measures of HPA-axis functioning: basal cortisol, cortisol awakening response (CAR) and anticipation before, reaction to, and recovery after a social stress task.

Measures of adversity

With regard to the adversity measures under study, we assume that a certain level of uncontrollability and unpredictability is conditional for adversity to have the potential to

affect the HPA-axis (Koolhaas et al., 2011; Miller et al., 2007). In addition, we expect that the association between adversity and changes in HPA-axis functioning can vary dependent on whether or not the event is a threat to the individuals integrity (Miller et al., 2007). Consequently, we will examine different types of adversity: 1) *social defeat*, including events that have a profound impact and threaten the individual's integrity like being a victim of abuse and 2) *loss/illness*, including events that also have a profound impact, but do not threaten the individuals integrity i.e., death and severe illness of a loved one.

Current study

In sum, we aim to 1) examine normative mean level changes and differential stability in HPA-axis functioning and 2) investigate whether and how adolescent adversity is related to deviations from normative changes in basal cortisol, the CAR and stress-induced cortisol responses. Normative increases are expected in both basal and stress induced cortisol. Given the more trait-like nature of basal cortisol compared to CAR (and maybe also stress-induced cortisol), larger differential stability is expected for basal cortisol than for the other measures. Furthermore, it is hypothesized that social defeat (but not loss/illness), is related to changes in HPA-axis functioning. With regard to the direction of the effects, overall down regulation of the stress system is expected after social defeat, that is, blunted basal cortisol, CAR and reaction to stress.

METHODS

Sample

Data were used from TRAILS (TRacking Adolescents' Individual Lives Survey), a large prospective cohort study of Dutch adolescents, who are followed biennially or triennially from 11 to at least 25 years of age. The present study involves a subset of 177 subjects from the TRAILS sample and data from the third and fourth assessment wave (described below). At wave 1, 2230 pre-adolescents (50.8% girls) enrolled in the TRAILS study (response rate 76.0%) of whom, 1816 (response rate 81.4%, 45.3% girls) participated in wave 3. At wave 3, the mean age was 16.13 years (SD = 0.59). At wave four 1881 adolescents (84.3%, 52.3% girls) participated with a mean age of 19.1 (SD = 0.60). A detailed description of the sample selection, procedures and methods can be found in De Winter and colleagues (de Winter et al., 2005).

Procedures

Procedure age 16. During the third wave, 744 adolescents were invited to participate in a series of laboratory behavioural experiments in addition to the usual assessments of whom 715 (96.1%) agreed to do so. Adolescents with at least one risk factor for mental health problems had a slightly greater chance of being selected for the experimental session (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). The risk factors were wave 1 temperament, lifetime parental psychopathology, and environmental risk. In total, 66.0%of the sample had one of

the above-described risk factors; the remaining 34.0% were selected randomly from the low-risk TRAILS participants. Nonetheless, the subsample selected for the current study did not differ significantly from the rest of the sample on any of the risk factors mentioned, probably due to non-random dropout across waves.

Procedure age 19. At wave 4 a subsample of adolescents was interviewed about life stress with the Life Stress Interview (LSI; Kendler, Karkowski, & Prescott, 1998). Because the life events interview was labour-intensive, it was only administered to part of the sample (n = 957). During the interview adolescents were asked about stressful events that occurred in the last two years. Events were rated by the interviewer, both with regard to the contextual severity of the event and with regard to person-dependency, on a 4-point scale.

Of the adolescents that were interviewed AND had participated in the behavioural experiments at age 16, a subsample was invited to participate in a reassessment of the behavioural experiments. After a first batch of LSI's had been collected norms were established for selecting adolescents into a no adversity group and two adversity groups. The first adversity group, the *social defeat* group, consisted of adolescents who had been a victim of bullying, sexual intimidation or violence or were dumped after a serious relationship. The second adversity group, the *loss* group, consisted of adolescents exposed to death and serious illness in the adolescents' close environment (e.g., family, close friends). An additional major criterion to be included in one of the two adversity groups was that the event was rated 3 or 4 at the contextual severity scale. The no-adversity group included a random selection of those adolescents who did not experience the target loss and defeat events. Eligible adolescents received a letter to invite them to participate in the behavioural experiments, including a social stress task (response rate 85.0%)

Social Stress Test. This test was the last challenge of the experimental session. It involved a standardized protocol including public speaking and mental arithmetic, inspired by the Trier Social Stress Task, for the induction of moderate performance- related social stress.

At age 16, the participants were instructed to prepare a 6-min speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged by a panel of peers after the experiment. The participants had to speak continuously for the whole period of 6 min. The speech was followed by a 3-min interlude in which the participants were not allowed to speak. After the interlude, participants were instructed to subtract 17 repeatedly, starting with 13.278. This difficult task was meant to induce a sense of uncontrollability.

At age 19, the test protocol was identical to the protocol at age 16 except two differences: 1) Adolescents were asked to present in front of a committee instead of a camera. Participants were told that the committee judged content of the presentation as well as gesture. 2) Instead of presenting about themselves and their lives, adolescents were instructed to convince the committee to either hire them for a job or select them for an educational program.

In total, 177 adolescents participated in the behavioural experiments both at age 16 and 19 (no stress = 67; social defeat = 32 and loss/illness = 62, 16 participants experienced both

social defeat and loss/illness and were included in both adversity groups). A previous study by Bouma and colleagues on the effects of gender, menstrual phase, and use of oral contraceptives in the same sample at age 16 indicated that oral contraceptive users showed a lower CAR and a blunted cortisol response to the social stress test (Bouma et al., 2009). Therefore, girls using oral contraceptives (n = 36) were excluded from all analyses. Consequently, 141 adolescents were included in the analyses of the current study (32,6% girls).

Measures

Basal cortisol and cortisol awakening response. To collect data on basal cortisol and CAR, both at age 16 and at age 19 participants received a verbal and written instruction to collect saliva at home immediately after waking up as they were still lying in bed (CM1) and 30 minutes after awakening (CM2), using the Sarstedt Salivette device (Nümbrecht, Germany). Directly after sampling, saliva samples were stored by participants in their freezer and brought to the institute as soon as possible.

Stress-induced cortisol. We assessed HPA-axis responses towards the GSST by five cortisol samples (referred to as CE1, CE2, CE3, CE4 and CE5). There is a delay of approximately 20 min between the production of cortisol by the adrenal glands and the detectability of representative levels of cortisol in saliva. CE1, reflecting anticipatory cortisol levels, was taken at the start of the experimental session. CE2 was collected just before the GSST, reflects HPA axis activity approximately 20 min earlier, and is considered a pre-test measure. CE3 was collected directly after the end of the GSST and reflects cortisol levels during speech. CE4 was collected 20 minutes after CE3 and reflects cortisol levels during arithmetic. CE5, collected 40 min after the end of the GSST reflects post-test cortisol level.

Other variables. Sex and habitual smoking (i.e., at least one cigarette a day) were included as potential confounders of the associations under study.

Statistical analyses

Analyses were done on complete cases (ranging from $n = 105$ for changes in Anticipation to $n = 132$ for changes in reactivity to stress; missing data were a result of failed saliva analyses or extreme values) and performed in SPSS (Version 18.0). We first calculated descriptive statistics of the (untransformed) variables used in this study, split for the three adversity groups. All cortisol variables were transformed before further analysis.

The standardized score of CM1, the first cortisol measure directly after awakening, was used as a measure of basal cortisol. The cortisol awakening response was calculated as CM2-CM1. Then, CAR scores were standardized to have a mean of 0 and a standard deviation of 1. The standardized score of CE1 was used as a measure of anticipation to stress. Reaction and recovery were calculated by saving the standardized residuals from regression analyses: 1) for reaction, stress task cortisol (speech, CE3) was predicted by the pre-test measure (CE2); 2) for recovery, post-test cortisol (CE5) was predicted by the task measure (CE3). Standardized

residuals are the residuals divided by an estimate of their standard deviation and have, similar to normal z-scores, a mean of 0 and a standard deviation of 1. Scores reflect the distance to the regression line and can consequently be used as a measure of individual differences in change during the experiments.

Normative changes were examined using repeated measures analyses including only the adolescents who were not exposed to social defeat or loss between age 16 and 19. Differential stability was assessed by means of test-retest correlations. To analyse associations between adversity and changes in HPA-axis functioning repeated measures analyses were performed including the total sample. Exposure to social defeat or illness was included as independent variables, gender and smoking as covariates.

RESULTS

Descriptive Statistics

Descriptive statistics of the untransformed cortisol variable, including means, standard deviations, age 16-age 19 correlations and differences between the three adversity groups are reported in Table 1. With regard to differences in untransformed cortisol variables between the adversity groups, adolescents exposed to social defeat showed a lower cortisol level after the stress task at age 19, but not at age 16, than adolescents not exposed to adversity.

Normative changes in HPA-axis functioning

Normative changes in basal, awakening and stress-induced HPA-axis functioning were assessed by means of repeated measures analysis in adolescents not exposed to adversity. We found significant increases in HPA-axis reactivity to the stress task from age 16 to age 19 ($F (1, 39) = 6.70$, $p = .013$). No changes were found in basal ($F (1, 39) = .10$, $p = .759$), CAR ($F (1, 39) = 1.54$, $p = .222$), anticipation ($F (1, 37) = .07$, $p = .798$) and recovery ($F (1, 42) = .13$, $p = .717$). As presented in Table 1, test-retest correlations showed modest differential stability in most cortisol measures except in reaction to the stress task, for which differential stability was substantial.

Adversity and changes in HPA-axis functioning

Associations between adversity and HPA-axis functioning were examined using repeated measures analyses in which exposure to social defeat or illness were included as independent variables. We found a significant effect of social defeat on changes in reaction to the stress task (Table 2; Figure 1). A borderline significant effect of social defeat was found on changes in basal cortisol. Loss was related to neither reaction nor to basal cortisol. Social defeat and loss were not associated with changes in the CAR, anticipation and recovery.

Table 1. *Descriptive characteristics for the raw cortisol variables.*

	No Adversity (n = 67)				Loss/illness (n = 78)					Social Defeat (n = 48)				
	M	***SD***	***n***	***r***	**M**	***SD***	***n***	***r***	**Loss vs. no advers.**	**M**	***SD***	***n***	***r***	**Defeat vs. no adversity**
Age 16														
CM1	8.17	4.68	40		8.68	4.79	44		t(82)= -.49, *p*=.625	7.64	4.43	21		t(59)= .43, *p*=.671
CM2	12.40	4.91	40		14.47	6.29	44		t(82)= -1.67, *p*=.099	13.45	5.54	21		t(59)= - .76, *p*=.450
Pre-experiments	3.28	1.85	43		4.10	5.22	47		t(87)= - .78, *p*=.437	4.40	1.56	25		t(65)= .26, *p*=.799
Pre-stress task	4.46	2.37	43		5.23	6.01	46		t(88)= - .97, *p*=.334	4.30	2.73	24		t(66)= - .26, *p*=.793
Stress (speech)	4.86	2.64	44		5.47	4.69	47		t(89)= - .76, *p*=.450	5.28	4.04	25		t(67)= - .53, *p*=.595
Stress (arithm.)	4.14	2.23	44		5.18	6.56	47		t(89)= - .99, *p*=.323	4.92	4.22	25		t(67)= - 1.01, *p*=317
Post-stress task	3.48	1.62	43		4.43	7.19	47		t(88)= - .85, *p*=.396	3.51	1.88	25		t(66)= - .07, *p*=.943
Age 19														
CM1	8.37	4.77	53	.169	8.58	5.13	64	.103	t(115)= - .24, *p*=.815	10.07	5.26	37	.230	t(88)= - 1.59, *p*=.115
CM2	15.54	6.92	53	-.012	15.97	7.39	64	.060	t(115)= - .32, *p*=.748	16.64	8.19	37	.142	t(88)= - .69, *p*=.495
Pre-experiments	5.76	3.37	53	.442	6.14	3.95	64	-.012	t(115)= - .56, *p*=.579	5.69	2.27	37	.220	t(88)= .11, *p*=.913
Pre-stress task	4.26	2.52	53	.147	4.76	3.18	64	.030	t(115)= - .94, *p*=.350	4.40	1.75	37	.294	t(88)= - .31, *p*=.759
Stress (speech)	8.57	5.07	53	.574	8.21	5.88	64	.412	t(115)= .35, *p*=.726	6.55	4.21	37	.303	**t(88)= 1.99, *p*=.050**
Stress (arithm.)	7.86	5.26	53	.475	7.36	4.87	64	.257	t(115)= .54 *p*=.592	6.16	4.22	37	.292	t(88)= 1.64, *p*=.105

Note: Descriptives for cortisol data reflect untransformed data. All cortisol measures are in nmol/l. r reflects the correlation coefficient between age 16 and age 19. **Bold** = significant association at $p < .05$.

Table 2. *Conditional repeated measures analyses.*

		F	DF(error)	*P*	η^2
Basal cortisol	Social Defeat	3.46	1(90)	.066	.037
	Loss/illness	.00	1(90)	.958	.005
CAR	Social Defeat	.08	1(84)	.780	.001
	Loss/illness	.31	1(84)	.580	004
Anticipation	Social Defeat	.04	1(98)	.833	.000
	Loss/illness	.06	1(98)	.802	.001
Reaction	Social Defeat	**6.30**	**1(97)**	**.014**	**.061**
	Loss/illness	.00	1(97)	.961	.000
Recovery	Social Defeat	1.48	1(98)	.226	.015
	Loss/illness	.06	1(98)	.814	.001

Note. Gender and smoking were included in all analyses as a covariate. **Bold** = significant association at $p < .05$.

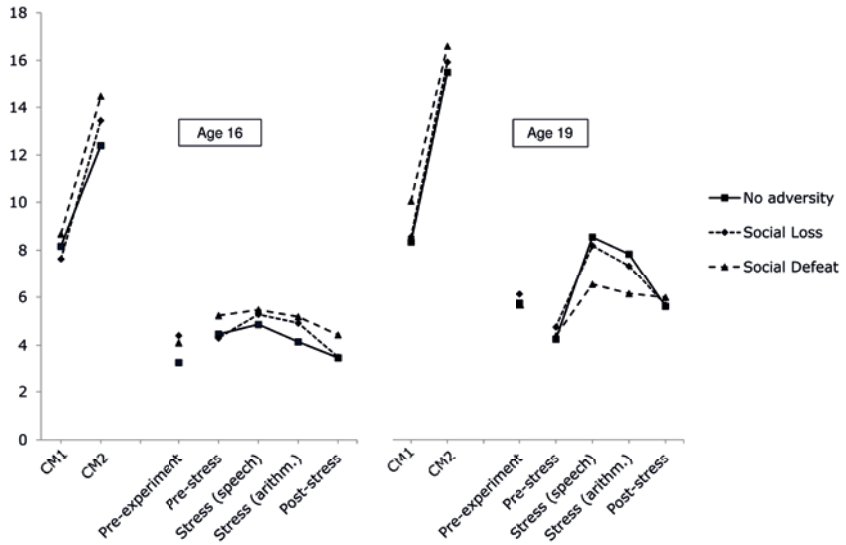

Figure 1. Graphic representations of the various cortisol measures, split for the three adversity groups, at age 16 and at age 19. The cortisol measures are the untransformed variables as presented in Table 1. Cortisol values are in nmol/l.

DISCUSSION

The current study provide unique longitudinal data about normative and adversity-driven changes in basal as well as social stress-induced HPA-axis functioning from age 16 to age 19. Data were used from 141 adolescents, a relatively large sample in the field of research into neuroendocrine responses to stress. First, we investigated normative mean level changes and

differential stability in basal cortisol, the CAR and stress-induced HPA-axis functioning. Second, we examined whether and how adversity was related to changes in basal, awakening and stress-induced cortisol responses. We found that increases in reaction to a social stress task were normative from age 16 to 19. Differential stability was modest to substantial. Adolescents who were exposed to social defeat did not show the normative increases in reaction to stress found in the other adolescents. Instead, they showed a comparable, moderate cortisol response at both ages. No normative changes were found with respect to basal cortisol, the CAR, anticipation to the stress task and recovery after the task, neither did we find effects of adversity on these measures.

Normative changes in HPA-axis activity.

Normative change was larger in reaction to the stress task compared to the other cortisol measures. With regard to basal cortisol, our findings seem to be in line with the findings by Trickett and colleagues suggesting that normative increases from childhood to adolescence are levelling off during late adolescence (Trickett et al., 2010). The substantial increases in reaction to the stress task are also in line with cross-sectional findings (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). Nonetheless, the increases we found were remarkably large. In addition to developmental increases, changes in the design of our social stress task from age 16 to age 19, might have further increased the HPA-axis reactivity. Because of expected test-retest effects, at age 19 adolescents were instructed to present in front of a jury instead of a camera. Presenting in front of jury is likely to elicit larger stress responses than presenting in front of a camera and may therefore countervail test-retest effects. Although we were unable to disentangle the proportion of increase due to changes in our design and due to maturation, taking together our findings and previously published cross-sectional studies, findings clearly point into the direction of normative increases in HPA-axis reactivity during adolescence.

Subsequently, we examined differential stability, the relative placement of individuals within a group. Studying differential stability of HPA-axis functioning is novel to the literature and can provide new insights in individual differences in maturation of the HPA-axis. Contrasting our hypothesis, differential stability of basal cortisol was not higher than stability of the other measures; instead, in particular stability of reaction to the stress task was substantial. Although longitudinal studies are scarce, it seemed plausible to expect higher stability in basal cortisol than in reaction to stress, given the different nature of the two. Basal cortisol has sometimes been suggested to be a relatively stable, trait-like characteristic (Hellhammer et al., 2007), whereas CAR (and maybe also stress induced cortisol) has been suggested to be more situation dependent, and can therefore be seen as a mainly state-like characteristic. The low test-retest correlations found for basal cortisol and CAR might be a result of relatively large measurement error. Morning cortisol was sampled at the participant's home, without the presence of a test-assistant. Adolescents may not have followed the written instructions strictly, resulting in limited resemblance between the settings at age

16 and 19, and subsequent low test-retest correlations. In contrast, stress-induced cortisol was measured in the lab, in a highly standardized setting, resulting in substantial similarity between the cortisol samples as measured during the stress-task at age 16 and as measured at age 19. Consequently, whereas basal cortisol might be a trait component (although sensitive to measurement error), stress-induced cortisol seems to be neither a typical state nor a typical trait component, which might explain the relatively high differential stability.

Adversity-driven changes in HPA-axis activity

Our finding that adolescents exposed to social defeat did not show the normative increase in reaction to stress shown by other adolescents, provides evidence that this type of adversity might have a long-term effect on HPA-axis functioning. Adolescents exposed to social defeat, but not loss, did not show normative increase in reaction to stress. In previous cross-sectional studies, integrity threatening events have been associated with blunted cortisol responses to stress (MacMillan et al., 2009; Ouellet-Morin et al., 2011). Our study is the first to confirm this association in a longitudinal design.

An explanation for the difference between social defeat and loss might be that the effects of social defeat are longer lasting than those of loss. Persisting high physiological stress can have damaging effects on brain structures (Carrion, Weems, & Reiss, 2007; Lupien, McEwen, Gunnar, & Heim, 2009). For example, it has been proposed that long-lasting increased basal cortisol levels, which can be caused by reduced recovery after acute stress exposure, have adverse effects on the cerebellum (De Bellis & Kuchibhatla, 2006). By reducing activity of the HPA-axis, the body can prevent this damage, which might explain the 'blunted' response after exposure to social defeat. So, even though a blunted basal cortisol and stress response has repeatedly, although not prospectively, been associated with psychopathology (Gunnar & Vazquez, 2001; Miller et al., 2007), a blunted response also protects the brain from damage, and may consequently have adaptive value. This is in line with the recent findings that a subgroup of the most psychosocially resilient maltreated youth had relatively low cortisol levels compared to other adolescents (Trickett et al., 2010). Taken together, more longitudinal prospective studies are needed to investigate whether the blunted responses are primarily adaptive and contribute to resiliency with regard to the development of psychopathology, or rather reflect a risk factor for mental and physical disorders.

Social defeat was related to changes in reaction to the social stress task, but not consistently to changes in the other measures of HPA-axis functioning. Adolescents exposed to social defeat showed a small increase in basal cortisol compared to other adolescents, although this effect was only borderline significant. Miller and colleagues provided evidence that integrity threatening events were related to lower, not higher, basal cortisol levels (Miller et al., 2007). It is not clear why we found the opposite but it should be noted that that 'being in the midst of a divorce' was related to higher levels of basal cortisol in Miller's study, not to lower basal cortisol like the other events. Nonetheless, given the small effect size and borderline significance, it might also be that our results on basal cortisol are a chance finding.

The larger effect of social defeat on reaction compared to basal cortisol may also be interpreted in terms of state and trait components, that is, reaction to stress has been suggested to be largely situation dependent and may consequently be more sensitive to environmental influences like stress. Although test-retest correlations did not show larger differential stability for basal cortisol than for the other cortisol measures, support for the difference between cortisol measures in terms of trait and state characteristics was found in our previous study, suggesting that basal cortisol, but not CAR or stress-induced cortisol, was related to personality facets (also trait-like characteristics; Laceulle, Nederhof, van Aken, & Ormel, Submitted). Although the difference between trait- and state components of HPA-axis functioning supports our findings on basal cortisol and reaction to stress, it does not explain the lack of association between adversity and the CAR, anticipation and recovery. Since the CAR has been suggested to reflect anticipation of demands of the upcoming day (Fries et al., 2009), it is, like reaction to stress, mainly influenced by state components and may therefore be likely to change following adversity. Also with regard to recovery after the stress task, reflecting perceived controllability, adversity driven changes seem plausible (Koolhaas et al., 2011). However, controllability may particularly decrease directly following the stressor, whereas the focus of the current study was on more long-term effects of adversity. Anticipation, finally, is an indication of predictability (Koolhaas et al., 2011), does not per se reflect the physiological capability to activate the stress system and may therefore be unlikely to be affected by the down regulation of the system after exposure to social defeat both at the short-term and at the long-term.

To conclude, this study is one of the first demonstrating that increases in stress-induced cortisol reactions are normative from age 16 to 19. New to the literature is also the focus on differential stability. Although differential stability was modest in most cortisol measures, substantial stability was found in reaction to stress. The current study provides unique longitudinal evidence that adolescent social defeat, but not loss or severe illness, can reduce the body's neuroendocrine response to social stress for, on average, a substantial period of time. This suggests that the potential of adverse events to affect HPA-axis functioning depends on whether or not events are integrity threatening.

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6

Stress-sensitivity and reciprocal associations between stressful events and temperament across adolescence

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Submitted

ABSTRACT

The current study had two aims: First, to elucidate the longitudinal, bidirectional associations between stressful events and temperament from childhood to late adolescence. Second, to investigate whether the immediate effects (within wave correlations) and delayed effects (stress à temperament paths) of stressful events on temperament were moderated by a cumulative plasticity gene index, prenatal adversity and by the combination of these two. Data were used from TRAILS, a large population cohort of Dutch adolescents. Temperament traits were assessed at 11, 16 and 19 years. Data of stressful events that occurred between age 0 and 11, between age 11 and 16, and between age 16 and 19 were captured using interviews. The results indicated that although stressful events and temperament traits are associated from childhood to adolescence, the direction of the effects depends on the temperament trait under study. In addition, correlations between stressful events and temperament were stronger in individuals high on both plasticity genes and prenatal adversity than in other individuals. Although these differences were not significant in the more conservative multi-group cross-lagged analyses, they point at the possibly important role of epigenetics in GxE studies.

INTRODUCTION

Exposure to major stressful life events during childhood, adolescence or adulthood is related to a heightened vulnerability to the development of mental disorders later in life (Rutter, 2006). In addition, stress might also affect more fundamental characteristics, like temperament (Laceulle, Nederhof, Karreman, Ormel, & Van Aken, 2012). Many studies start from a stress-effect model, assuming that stress influences temperament, and thus disregard the role of temperament in subsequent stress exposure. Other studies however suggest that certain traits may also evoke exposure to stressful events (e.g., Neyer & Lehnart, 2007). The current study disentangles longitudinal reciprocal effects between stressful events and temperament during adolescence. Additionally, individual characteristics may make certain individuals more sensitive to the influence of stressful events (Belsky, 1997). Therefore, the second focus of this paper is on sources of sensitivity to stressful events; putative plasticity genes, the prenatal environment and their interaction.

Stressful events and temperament

Over the last few years, increasing support has been found for the notion that exposure to stressful events might be related to changes in temperament and personality traits. More specific, adverse events have been found to predict increases in emotional instability over a multiple year period (Costa Jr, Herbst, McCrae, & Siegler, 2000; Laceulle et al., 2012; Löckenhoff et al., 2008; Mroczek & Spiro, 2003; Vaidya, Gray, Haig, & Watson, 2002). These findings on emotional instability tend to be highly consistent across studies, both those focusing on extremely adverse events and those on milder events. Only a few studies investigated stress effects on other traits, suggesting stronger influences on emotional instability than on other traits such as extraversion and conscientiousness (Laceulle et al., 2012; Löckenhoff et al., 2008).

Of all traits, emotional instability has been found to correlate strongest and most consistent with psychopathology (Kotov, Gamez, Schmidt, & Watson, 2010). Given the well investigated association between stressful events and (future) psychopathology (e.g., depression, Kendler, Karkowski, & Prescott, 1998); it seems plausible that stressful events can also cause changes in emotional instability. Nonetheless, stressful events do not correlate exclusively with emotional instability. Cross-sectional studies have suggested that exposure to stressful events might be related to elements of extraversion and conscientiousness, other traits that, like emotional instability, have often been associated with psychopathology. For example, in a cross-sectional study, positive correlations have been reported between extraversion and mild negative life-events (Farmer et al., 2002). In a previous longitudinal study we found that adolescents exposed to stressful events showed slightly less maturation (i.e., increases) in traits related to both extraversion and conscientiousness (Laceulle et al., 2012). Other longitudinal studies however, reported no change in emotional instability, extraversion or conscientiousness after exposure to stressful life events (Löckenhoff et al., 2008; Specht, Egloff, & Schmukle, 2011). The apparent differences across studies and between temperament traits may indicate that the

nature of the associations differs dependent on the traits under study as well as on the study design (e.g., cross-sectional vs. longitudinal).

Direction of associations between stressful events and temperament

So far, several longitudinal studies between stressful events and emotional instability departed from a stress-effect model. The stress-effect model assumes that stressful events affect temperament change and disregards the possibility that temperament can also affect subsequent stress exposure. In contrast, the association between neutral or normative life events and temperament, such as getting married or becoming a parent, has mainly been studied from the perspective of a trait-effect model, with temperament as a predictor of life events. For example, individuals high on extraversion were more likely to start their first romantic relationship or move in together, than adolescents low on extraversion (Neyer & Lehnart, 2007; Specht et al., 2011). This seems to suggest that whereas stressful events might predict temperament change (in particular emotional instability), temperament (in particular extraversion) might predict normative events. Additionally, some evidence suggests that temperament can predict stressful events as well. For example, high extraversion and conscientiousness were related to less subsequent stressful life-events (Lüdtke, Roberts, Trautwein, & Nagy, 2011), and reciprocal causation between stressful life events and emotional instability was found in a prospective twin study (Middeldorp, Cath, Beem, Willemsen, & Boomsma, 2008). However, in a two-wave study examining the causal pathways between life events and emotional instability (i.e., neuroticism) and between life events and extraversion, high emotional instability (but not extraversion) predisposed people to experience more stressful life events whereas life events were not found to predict later temperament (Magnus, Diener, Fujita, & Pavot, 1993).

Taken together, it seems clear that at least for traits related to emotional instability, the association with stressful events can be bidirectional. Given the weak predictive power of stressful events regarding changes in extraversion and conscientiousness (Laceulle et al., 2012; Löckenhoff et al., 2008), it might be that the associations that are reported between stress and these traits are best accounted for by a trait-effect model instead of either a stress-effect or a bidirectional model. Investigating fully recursive models is the only way to disentangle which model best accounts for the association between stressful events and temperament (Neyer & Asendorpf, 2001). Therefore, the first aim of the current study is to investigate the associations between stressful events and five temperament traits using cross-lagged structural equation models with three waves of data. Temperament traits under study were elements of emotional instability, extraversion and conscientiousness, the three traits that have been consistently linked to psychopathology (Kotov et al., 2010). Two traits related to emotional instability were included (fear and frustration), two traits related to extraversion (affiliation and shyness) and one trait related to conscientiousness (effortful control). We hypothesize that fear and frustration are associated with stressful events in a bidirectional way, whereas affiliation, shyness and effortful control affect stressful events more than vice versa (trait-effect model).

Individual differences in sensitivity to stressful events: putative plasticity genes, early environment and their interaction

Over the last decade an increasing emphasis has emerged on the possibility that individual characteristics make some individuals consistently more sensitive to stressful events than others (Belsky & Pluess, 2009). Genetic characteristics as a source of differential sensitivity to the environment (GxE) can be considered a trending topic. For example, the moderating effect of the short allele in the serotonin transporter promotor region (5-HTTLPR) on the association between stress and depression reported by Caspi and colleagues has been cited over 2,900 times (Caspi et al., 2003). Recent evidence shows that the same allele also increases sensitivity to positive parenting (Hankin et al., 2011). Thus, individuals with a certain genotype may be more sensitive to influences from the environment, for better and for worse.

Until now, there are numerous published attempts at replicating GxE findings, and extending them to other genes and/or outcome measures. These studies have resulted in a mixed pattern of findings, suggesting 1) that several genes, so called plasticity genes, might be responsible for differences in stress sensitivity and 2) that it is difficult to replicate the associations found for a specific single gene (Munafò, Durrant, Lewis, & Flint, 2009). One of the factors that might be responsible for the lack of replication may simply be a lack of power. Most plasticity genes have only small effects and some occur only in a small percentage of the general population. Recently, the use of a cumulative genetic plasticity index has been proposed, in which sensitivity to the environment is hypothesized to increase with increasing numbers of plasticity alleles (Belsky & Pluess, 2009; Belsky & Beaver, 2011; Stavrakakis et al., 2012). Using a cumulative plasticity index might solve the problem of power regarding plasticity genes.

However, other factors are likely to be responsible for the lack of replication as well, of which the most challenging is probably gene expression. Parallel to the study of candidate genes, an increasing emphasis has emerged on epigenetics. Shortly, epigenetics is the study of gene transcribability, that is, whether genes are activated (or 'turned on') and able to induce the production of the proteins that affect behaviour. Accordingly, when studying one of the proposed plasticity genes, it might be that two individuals with the same allele, do not show the same behaviour because the gene is turned on in one individual and turned off in the other. Whether genes are turned on or off is regulated by the epigenome, a complex biochemical system that changes gene activity without changing the gene itself (Bernstein, Meissner, & Lander, 2007). In addition, increasing evidence has emerged for the interplay between the genome and the environment in the activation of genes. For example, associations between 5-HTTLPR polymorphisms and psychological problems have been suggested to be are altered by environmentally influenced DNA methylation patterns, a process associated with epigenetics (van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010). This (de)activation of genes is an ongoing process, essential to normal development (Charney, 2012). However, the literature suggests that the prenatal period is an important period for environmental influences on gene activation, or, as noted by Ollikainen

and colleagues (Ollikainen et al., 2010, p. 4176), "… a sensitive time for the establishment of epigenetic variability in humans, with implications for the effects of maternal environment in addition to genetics on the development of the newborn epigenome and potentially for programming of later disease risk".

Therefore, it seems plausible that the prenatal environment plays a role in the association between genetic characteristics and later stress-sensitivity. Several studies have indeed suggested that the prenatal environment interacts with plasticity genes in pathways to psychopathology (e.g., Pluess et al., 2011; Wakschlag et al., 2010), although as test of heightened sensitivity has never been reported in the literature. In addition to the role of epigenetics, the prenatal environment has consistently been found to have profound and long-term influences on development (e.g., Harris & Seckl, 2011; O'Connor, Heron, Golding, Glover, & the ALSPAC Study Team, 2003), although no evidence has been found so far for enhanced sensitivity to childhood stressful events following prenatal adversity in humans (Laceulle et al., 2013). Therefore, the current study aims to examine genetic and prenatal adversity, as well as the interaction between the two, as potential sources of individual differences in sensitivity to the influence of later stressful events on immediate and subsequent temperament traits. We hypothesize that the impact of stressful events on subsequent temperament is stronger in adolescents with both a large number of plasticity genes and high levels of prenatal adversities compared to adolescents with none or either one sensitivity factor. Thus, we will only test sensitivity effects with regard to the within wave correlations between stressful and temperament and the paths from stressful events to subsequent temperament. It might be tempting to test whether there is also something like a sensitivity effect for the influence of temperament on subsequent stress. However, the number of research questions as well as the subsequent number of analyses is already considerable, and since we did not have strong hypotheses for differences in sensitive to temperament we decided to limit ourselves to the current research questions.

In summary, the current study has two major aims: First, we will elucidate longitudinal reciprocal effects between stressful events and temperament from childhood to late adolescence. Second, we aim to investigate whether the immediate and delayed impact of stressful events on temperament is moderated by plasticity genes (G), prenatal adversity (i.e., pregnancy and delivery adversity, PDadv) and by the interaction between the two (GxPDadv).

METHODS

Sample

The TRacking Adolescents' Individual Lives Survey (TRAILS) is a large prospective cohort study of Dutch adolescents, who are followed biennially or triennially from 11 to at least 25 years of age (Ormel et al., 2012). The present study involved data from the first, third and fourth assessment wave. A detailed description of the sample selection, procedures and methods can be found in De Winter and colleagues (de Winter et al., 2005). At the start of the project,

the target sample involved all 10 to 11year-old children living in the north of the Netherlands, in both cities and rural areas. Selected municipalities were requested to give out names and addresses of all children of the target group (3483 names). At the same time, schools were asked to participate. School participation was a prerequisite for children and parents to be asked to enrol in the study. Of the 135 primary schools in the area, 90.4% of the schools, accommodating 90.3% of the children, agreed to participate. Then, both parents and children were asked for an agreement to participate, resulting in 2.230 participants. At wave 1, the mean age of the adolescents enrolled in the study was 11.09 years (SD = 0.56). At wave 3, the mean age was 16.13 years (SD = 0.59) and at wave 4 the mean age was 19.1 (SD = 0.60).

Measures

Temperament. Child temperament was assessed at ages 11, 16 and 19 years by means of the short form of the parent version of the Early Adolescent Temperament Questionnaire-Revised (EATQ-R, Hartman, 2000; Putnam, Ellis, & Rothbart, 2001). We used the parent version as in our sample; the factor structure of this version was superior to that of the child version at age 11 (Oldehinkel, Hartman, de Winter, Veenstra, & Ormel, 2004). Because the scales as proposed by Rothbart and co-workers had not been verified empirically in large population samples, principal component analysis was used to investigate the extent to which the original scales reflected the structure of the EATQ-items in the TRAILS sample. This led to some minor alterations of the original scales (Oldehinkel et al., 2004). The following five scales were distinguished: (i) fear (negative affect related to anticipated pain or distress, five items, Cronbach's α = .63); (ii) frustration (negative affect related to interruption of ongoing tasks or goal blocking, five items, α = .74); (iii) shyness (slow or inhibited approach and/or discomfort in social situations, four items, α = .84); (iv) effortful control (capacity to control attention, activation and inhibition, 11 items, α = .86) and (v) affiliation (desire for, and pleasure in, warmth and closeness with others, six items, α = .66). Answers were rated on a five-point Likert-type scale (1 = 'almost always untrue' to 5 = 'almost always true'). Higher values indicated a higher presence of the temperamental trait concerned. Missing items were imputed by means of Corrected Item Mean imputation (CIM; Huisman, 2000). Test–retest stability of the EATQ-R scales has been found to be moderate to good, ranging from .69 to .85 across scales (Muris & Meesters, 2009).

Life events. Stressful events were measured using various interviews, assessing life events that may have brought changes to their life and that occurred since the previous assessment. This resulted in 3 waves of life event data covering events that occurred from birth-age 11, age 11-16 and age 16-19. At the first wave, data were obtained from the mother using the TRAILS Family History Interview (Nederhof et al., 2010). At the other two waves, data were obtained from the adolescent using respectively the Event History Calendar (Caspi et al., 1996) and the Life Stress Interview (Kendler et al., 1998). Events were included that have previously been found to be likely to be experienced as stressful and bring change to someone's life, e.g., death or illness of a family member, or close friend, parental divorce and being a victim of bullying

of violence (McMahon, Grant, Compas, Thurm, & Ey, 2003). Given the difference in time span for the childhood events, early adolescent events and late adolescent events, and because some of the events are more likely to occur at certain ages than at others, sum scores were transformed into z-scores.

Prenatal adversity. Prenatal adversity was assessed during the first assessment wave with the TRAILS Family History Interview. The variable prenatal adversity reflected both pregnancy and delivery adversities (PDadv) and was created based on questions about maternal prenatal smoking, maternal prenatal alcohol use, birth weight, gestational age, and pregnancy and delivery complications (Nederhof et al., 2010). For birth weight, prenatal smoking, and pregnancy and delivery complications, the same criteria were used as Buschgens and colleagues did (Buschgens et al., 2009). For maternal prenatal alcohol use three groups were created: no alcohol use, mild alcohol use (up to three glasses per week) and heavy alcohol use (four glasses per week or more). Gestational age was also recoded into two groups: normal (between 34 and 42 weeks) and abnormal (33 weeks or less, or more than 42 weeks). The sum score of these variables (ranging from 0 to 6) was re-coded into a dummy variable indicating whether children were low/moderate or high on PDadv. There is no well-established clinical cut-off for this measure; we therefore identified high PDadv the top 15% (3-6 events).

Plasticity genes. Plasticity genes were defined as previously in the TRAILS sample (Stavrakakis et al., 2012). More specific, following the criteria set by Belsky and Pluess (2009), the A1 allele of DRD2, the long version of DRD4 (7 to 10 repeats), the short allele haplotype (5-HTTLPR) of SLC6A4, the 2R/3R alleles of MAOA, the A allele of TPH1, the T allele of the 5-HTR2A, the val(G) allele of COMT and the val66met allele of BDNF were defined as plasticity alleles. Blood samples (n=1190) or buccal swabs (Cythobrush®) (n=275) were collected for DNA extraction using a manual salting out procedure as described by Miller and colleagues (Miller, Dykes, & Polesky, 1988). The *BDNF* single nucleotide polymorphism (rs6265), *DRD2*/TaqIA (rs1800497), *COMT*/val158met (rs4680), *TPH1* (rs179913) and *5-HTR2A* (rs6313) were genotyped on the Golden Gate Illumina BeadStation 500 platform (Illumina, San Diego, California) according to the manufacturer's protocol. Call rates were: 81% for *BDNF*, 100% for *DRD2*, 95% for *COMT*, 100% for *TPH1* and 100% for *5-HTR2A*. All DNA samples could be amplified and concordance between DNA replicates (n=53) showed a 100% genotyping accuracy (Nederhof et al., 2010; Malouff, Thorsteinsson, & Schutte, 2005). Data cleaning was completed according to recommended procedures (Steptoe, Nolte, McCaffery, & Schnieder, 2010). All SNPs were well within Hardy-Weinberg equilibrium, with HWE p-values ranging between 0.42 and 0.52.

Genotyping of the length polymorphisms (LP) *DRD4, MAOA, HTTLPR* and SNP rs25331 (A/G SNP in *L HTTLPR*) was done at the Research lab for Multifactorial Diseases within the Human Genetics department of the Radboud University Nijmegen Medical Centre in Nijmegen, The Netherlands. Genotyping of the 5-*HTTLPR* in the *SLC6A4* (*5-HTT, SERT*) gene was performed by simple sequence length analysis. Call rate was 91.6%. A custom-made TaqMan assay (Applied Biosystems) was utilized in order to genotype the single nucleotide substitution (A to G) which is present in the *HTTLPR* long (l) allele (rs25531). Call rate was 96.5%. Concordance between

DNA replicates showed an accuracy of 100%. All *lg* alleles were recoded into *s'*, because it has been shown that this polymorphism represents low serotonin expression comparable to the *s'* allele, while *la* was recoded as *l'*. The 48 bp direct repeat polymorphism in exon 3 of *DRD4* was genotyped on the Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA, USA). Three percent blanks as well as duplicates between plates were taken along as quality controls during genotyping. Determination of the length of the alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) using standard conditions. Call rate for *DRD4* was 99.4%. The 30bp variable number of tandem repeat polymorphism (called *MAOA-LPR* or *MAOA-uVNTR*) was also genotyped on the Illumina BeadStation 500 platform (Illumina Inc., San Diego, CA, USA). Three percent blanks as well as duplicates between plates were taken along as quality controls during genotyping. Call rate was 100% for *MAOA*. All polymorphisms were well within Hardy-Weinberg equilibrium (HWE p-values ranged from 0.77 to 0.87).

Each polymorphism was assigned one point if a plasticity genotype was present and these values were summed to create a cumulative index. Girls who were heterozygous for the *MAOA* gene, which is located on the X chromosome, were categorized into the low activity (i.e., high plasticity) genotype as proposed by Belsky and Beaver (2011). There is no well-established cut-off for high genetic plasticity. Therefore we identified high genetic plasticity in line with a previous TRAILS study (Stavrakakis et al., 2012), that is, as the top 15% (6-8 genes).

Analyses

Analyses were performed within Mplus (Muthen & Muthen, 2007). We examined the research questions with cross-lagged path analyses using 3 waves of data (TRAILS wave 1, 3 and 4; we left out wave 2 because temperament was not assessed during that wave). In all analyses, missing values were estimated using Full Information Maximum Likelihood.

First, for all adolescents together, we tested a baseline model in which stability paths of temperament and stressful events, as well as within wave correlations (i.e., the concurrent initial relation between temperament and stressful events at age 11 and correlated change between them at age 16 and 19) were estimated (Table 1, model 1). Second, we tested a model with cross-lagged paths both from stressful events to temperament and from temperament to stressful events added to the baseline model (Table 1, model 2). Third, we tested whether respectively stability across waves of temperament (e.g., fear age 11 à fear age 16 = fear age 16 à fear age 19; Table 1, model 3), stability across waves of stressful events (Table 1, model 4) and cross-lagged paths (Table 1, model 5) could be constrained to be equal across waves. For reasons of parsimony, a model in which paths are constrained is desirable. However, since we used different measures of stressful events, the intervals between the waves differ substantially and because the association between stressful events and temperament might differ across development, it may not be possible to constrain all paths to be equal across waves.

Table 1. *Model fit comparisons.*

	Model fit indices				Model comparison test			
	χ^2	Df	CFI	RMSEA		$\Delta\chi^2$	Δdf	p
Fear								
M1 Stability and within wave	19.95	6	.982	.032				
M2 M1 + Cross lagged paths	2.17	2	1.00	.006	2 vs. 1	17.78	4	.001
M3 M2 + Fear paths constrained across waves	**2.48**	**3**	**1.00**	**.000**	**3 vs. 2**	**0.31**	**1**	**.578**
M4 M2 + Stress paths constrained across waves	6.10	3	.997	.018	4 vs. 2	3.93	1	.047
M5 M2 + Cross-lagged and within wave constrained	13.04	5	.990	.027	5 vs. 2	10.87	3	.012
Frustration								
M1 Stability and within wave	28.71	6	.979	.041				
M2 M1 + Cross lagged paths	1.52	2	1.000	.000	2 vs. 1	27.19	4	<.001
M3 M2 + Frustration paths constrained across waves	9.10	3	.994	.030	3 vs. 2	7.58	1	.006
M4 M2 + Stress paths constrained across waves	6.11	3	.998	.018	4 vs. 2	4.59	1	.032
M5 M2 + Cross-lagged and within wave constrained	**6.40**	**5**	**.999**	**.011**	**5 vs. 2**	**4.88**	**3**	**.181**
Affiliation								
M1 Stability and within wave	18.66	6	.989	.031				
M2 M1 + Cross lagged paths	.78	2	1.000	.000	2 vs. 1	17.88	4	.001
M3 M2 + Affiliation paths constrained across waves	25.78	3	.980	.058	3 vs. 2	25.00	1	< .001
M4 M2 + Stress paths constrained across waves	5.35	3	1.000	.007	4 vs. 2	4.57	1	.033
M5 M2 + Cross-lagged and within wave constrained	**7.74**	**5**	**.998**	**.016**	**5 vs. 2**	**6.96**	**3**	**.073**
Shyness								
M1 Stability and within wave	28.26	6	.984	.041				
M2 M1 + Cross lagged paths	2.40	2	1.000	.010	2 vs. 1	25.86	4	<.001
M3 M2 + Shyness paths constrained across waves	13.16	3	.993	.039	3 vs. 2	10.76	1	.001
M4 M2 + Stress paths constrained across waves	7.00	3	.999	.017	4 vs. 2	4.6	1	.032
M5 M2 + Cross-lagged and within wave constrained	**4.39**	**5**	**1.000**	**.000**	**5 vs. 2**	**1.99**	**3**	**.158**
Effortful Control								
M1 Stability and within wave	21.79	6	.990	.035				
M2 M1 + Cross lagged paths	2.43	2	1.000	.010	2 vs. 1	19.36	4	.001
M3 M2 + Eff. control paths constrained across waves	24.46	3	.987	.057	3 vs. 2	22.03	1	<.001
M4 M2 + Stress paths constrained across waves	6.46	3	.999	.019	4 vs. 2	4.03	1	.045
M5 M2 + Cross-lagged and within wave constrained	**3.60**	**5**	**1.000**	**.000**	**5 vs. 2**	**1.17**	**3**	**.279**

Note. M refers to Model. Best fitting model presented in **bold**.

Fit of the different models was compared using chi-square difference tests as well as change in comparative fit index (CFI) and the root mean square of error of approximation (RMSEA), with relatively lower RMSEA's and higher CFI's indicating better fit (Kline, 2005). Overall, values above 0.95 for the CFI and values lower than .05 for the RMSEA are an indication of adequate model fit (Hu & Bentler, 1999).

Fourth, for the model that fitted the data best, we used multi-group analyses to test moderation effects of prenatal adversities, plasticity genes, and the combination of these two on the effect of stressful events on temperament (within wave correlations and stress à temperament paths). We evaluated which model fitted the data best: a) a model without subgroups, b) a model differentiating between adolescents high on plasticity genes, but low/intermediate on PDadv versus all other participants, c) a model differentiating between adolescents high on PDadv but low/intermediate on plasticity genes versus all other participants and d) a model differentiating between adolescents high on both PDadv and plasticity genes versus all other participants. Based on our hypotheses that plasticity genes, PDadv and/or GxPDadv would predict increased sensitivity for the influence of stressful events on temperament, we only tested moderation effects for the within-wave correlations and for the cross-lagged paths from stressful events to subsequent temperament. If model fit was significantly better when differentiating between groups, we used model comparisons of specific paths in specific groups to determine which paths significantly differed from each other.

RESULTS

Descriptive statistics

Means and standard deviations on temperament and stressful events are reported in Table 2.

Table 2. *Descriptive statistics. Means and standard deviations of the variables under study.*

		Minimum	Maximum	Mean	SD
Stress	Age 0-11	-.82	4.84	0	1
(z-score)	Age 11-16	-.84	4.36	0	1
	Age 16-19	-1.33	3.16	0	1
Fear	Age 11	1.00	5.00	2.42	.73
	Age 16	1.00	4.40	1.94	.64
	Age 19	1.00	4.25	1.99	.62
Frustration	Age 11	1.00	4.80	2.78	.66
	Age 16	1.00	5.00	2.71	.69
	Age 19	1.00	5.00	2.35	.71
Affiliation	Age 11	1.50	5.00	3.87	.56
	Age 16	1.30	5.00	3.68	.63
	Age 19	1.00	5.00	3.54	.59

Table 2. *Descriptive statistics. Means and standard deviations of the variables under study.*

		Minimum	Maximum	Mean	SD
Shyness	Age 11	1.00	5.00	2.51	.89
	Age 16	1.00	5.00	2.35	.92
	Age 19	1.00	5.00	2.05	.84
Effortful	Age 11	1.09	5.00	3.22	.68
Control	Age 16	1.27	5.00	3.20	.66
	Age 19	1.11	5.00	3.23	.72

Cross-influences between stressful events and temperament traits

Fear. Fit of the baseline model with stability and within wave correlations was reasonable to good (Table 1, model 1, CFI and RMSEA). Adding cross-lagged paths to the baseline model with only stability paths and within wave correlations significantly improved model fit (Table 1, model 1 vs. model 2). For fear, the temperament paths could be constrained, indicating that the association between fear at age 11 and 16 was similar to the association between ages 16 and 19 (Figure 1). The stressful-events paths and the cross-influence paths could not be constrained, meaning that the strength of these paths differed significantly across waves. The path from stressful events to fear was weaker during late adolescence ($\beta = .04$) than at the other to ages ($\beta = .09$). Being exposed to more stressful events significantly predicted higher subsequent fear, whereas fear did not predict subsequent stressful events. The concurrent initial relation (within wave correlation age 11) was positive ($\beta = .13$), revealing that higher levels of fear were related to exposure to more stressful events. Correlated change (within wave correlations age 16 and 19), showed that increases in stressful events were related to increases in fear and vice versa (respectively $\beta = .17$ and .04).

Frustration. Fit of the baseline model with stability and within wave correlations was reasonable to good (Table 1, model 1, CFI and RMSEA). Adding cross-lagged paths significantly improved the model fit (Table 1, model 1 vs. model 2). Stability in both frustration and stressful events was higher during early adolescence than during late adolescence. For frustration the cross-influence paths could be constrained to be equal, indicating that the effects of stressful events at age 11 on temperament at age 16 and vice versa were equal to those from ages 16 to 19 (Figure 2). Being exposed to more stressful events significantly predicted higher levels of subsequent frustration, whereas frustration did not predict subsequent stressful events. The concurrent initial relation (within wave correlation age 11) was positive, revealing that higher levels of frustration were related to exposure to more stressful events. Correlated change (within wave correlations age 16 and 19), showed that increases in stressful events were related to increases in frustration and vice versa.

Affiliation. Fit of the baseline model with stability and within wave correlations was reasonable to good (Table 1, model 1, CFI and RMSEA). Adding cross-lagged paths significantly improved the model fit (Table 1, model 1 vs. model 2). Stability in both affiliation and stressful events

was higher during early adolescence than during late adolescence. For affiliation the cross-influence paths could be constrained to be equal, indicating that the effects of stressful events at age 11 on affiliation at age 16 and vice versa were equal to those from ages 16 to 19 (Figure 3). Adolescents high on affiliation were more likely to experience subsequent stressful events, but stressful events did not predict subsequent affiliation. Within wave correlations (the concurrent initial association at age 11 and the correlated change at age 16 and 19) were not significant.

Shyness. Fit of the baseline model with stability and within wave correlations was reasonable to good (Table 1, model 1, CFI and RMSEA). Adding cross-lagged paths significantly improved the model fit (Table 1, model 1 vs. model 2). Stability in both shyness and stressful events was higher during early adolescence than late adolescence. For shyness the cross-influence paths could be constrained to be equal, indicating that the effect of stressful events at age 11 on shyness at age 16 and vice versa were equal to those from ages 16 to 19 (Figure 4). Adolescents high on shyness were less likely to be exposed to subsequent stressful events, but stressful events did not predict subsequent shyness. Within wave correlations (the concurrent initial association at age 11 and the correlated change at age 16 and 19) were not significant.

Effortful control. Fit of the baseline model with stability and within wave correlations was reasonable to good (Table 1, model 1, CFI and RMSEA). Adding cross-lagged paths significantly improved the model fit (Table 1, model 1 vs. model 2). Stability in effortful control was higher during late adolescence compared to early adolescence, whereas stability in stressful events was higher during early adolescence than late adolescence. For effortful control the cross-influence paths could be constrained to be equal, indicating that the effect from stressful events at age 11 on effortful control at age 16 and vice versa were equal to those from ages 16 on 19 (Figure 5). Adolescents high on effortful control were less likely to be exposed to subsequent stressful events. The cross-lagged paths from stressful events to effortful control were significant, however, the magnitude of these paths was small ($\beta = .04$). The concurrent initial relation (within wave correlation age 11) was negative, revealing that lower levels of effortful control were related to exposure to more stressful events. Correlated change (within wave correlation age 16 and 19), showed that increases in stressful events were related to decreases in effortful control and vice versa.

Moderation effects in the association between stressful events and temperament

Differences in sensitivity to stressful events between 1) adolescents low on plasticity genes and low on PDadv, 2) children high on plasticity genes only, 3) adolescents high on PDadv, and 4) adolescents high on both plasticity genes and PDadv (GxPDadv), were explored by comparing bivariate correlations between the four groups (Table 3). Differences were tested both with regard to within wave, and with regard to stress à future temperament associations. The four groups differed substantially in size: for individuals low on plasticity genes and PDadv n = 1288; high on plasticity genes, but low on PDadv n = 274; low on plasticity genes, but high on

PDadv n = 255; and high on plasticity genes and high on PDadv n = 34. Almost all correlations between stressful events and fear, as well as a few other correlations, were significantly stronger in the GxPDadv group, suggesting that the strength of the associations between stressful events and (subsequent) temperament differed between the groups. Children high on either plasticity genes, or PDadv did not show consistently higher associations.

Moderation effects of plasticity genes (G, n = 274). Multi-group analyses comparing individuals low/intermediate and high on plasticity genes showed no moderation of plasticity genes on the within-wave and cross-lagged paths in any of our temperament traits (respectively, for fear $\Delta\chi^2 = 3.49$, $\Delta df = 5$, $p = .625$, for frustration $\Delta\chi^2 = 4.98$, $\Delta df = 3$, $p = .175$, for affiliation $\Delta\chi^2 = 1.58$, $\Delta df = 3$, $p = .664$, for shyness $\Delta\chi^2 = .36$, $\Delta df = 3$, $p = .948$ and for effortful control $\Delta\chi^2 = .95$, $\Delta df = 3$, $p = .815$).

Moderation effects of prenatal adversity (PDadv, n=255). Multi-group analyses showed that the associations between stressful events and frustration differed between individuals low/intermediate on PDadv and individuals high on PDadv (frustration, $\Delta\chi^2 = 16.81$, $\Delta df = 3$, $p = .001$). However, for frustration, only the within wave correlation at wave 1 differed significantly between adolescents high on PDadv and the other adolescents ($\Delta\chi^2 = 14.65$, $\Delta df = 1$, $p < 001$). No moderation of PDadv was found for the associations between stress and fear, affiliation, shyness and effortful control (fear $\Delta\chi^2 = 7.4$, $\Delta df = 5$, $p = .193$, affiliation $\Delta\chi^2 = .4$, $\Delta df = 3$, $p = .940$; shyness $\Delta\chi^2 = 0.61$, $\Delta df = 3$, $p = .894$; effortful control $\Delta\chi^2 = 4.27$, $\Delta df = 3$, $p = .234$).

Moderation effects of both plasticity genes and PDadv (GxPDadv, n=34). Despite the strong bivariate correlations in individuals with high GxPDadv, multi-group analyses showed that the associations between stressful events and temperament did not differ significantly between individuals low/intermediate on GxPDadv with high GxPDadv (fear $\chi^2 = 8.92$, $\Delta df = 5$, $p = .122$; frustration $\Delta\chi^2 = 1.59$, $\Delta df = 3$, $p = .661$; affiliation $\Delta\chi^2 = 7.15$, $\Delta df = 3$, $p = .067$, shyness $\Delta\chi^2 = 6.1$, $\Delta df = 3$, $p = .107$, and effortful control $\Delta\chi^2 = 6.61$, $\Delta df = 3$, $p = .085$).

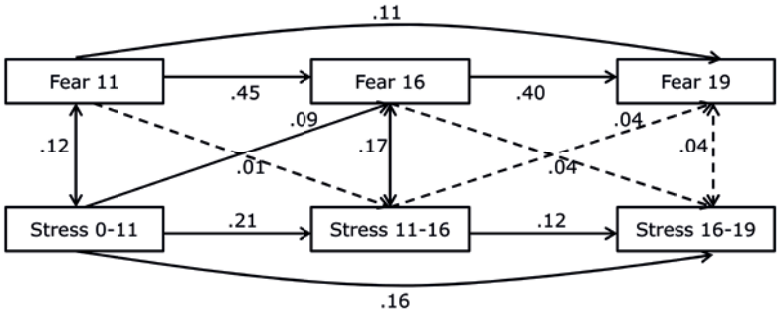

Figure 1. Fear. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. * = path coefficient (beta) < .05.

Table 3. *Bivariate correlations between stressful events and immediate and subsequent temperament, split for the different sensitivity groups.*

		Low G + Low PDadv =1288			High G N=274			High PDadv N=255			GxPDadv N=34		
		Stressful events			Stressful events			Stressful events			Stressful events		
		Age 0-11	Age 11-16	Age 16-19	Age 11	Age 16	Age 19	Age 0-11	Age 11-16	Age 16-19	Age 11	Age 16	Age 19
Fear	11	.10			.06			.16			.30		
	16	.12	.15		.06	.20		.17	.13		.42[ab]	.44[a]	
	19		.09	.09		.02	.19		.13	.10		.49[ab]	.49[ab]
Frustration	11	.05			.06			.28[ab]			.26		
	16	.09	.14		.09	.17		.18	.22		.29	.28	
	19		.15	.11		.13	.15		.13	.14		.32	.35
Affiliation	11	-.01			.06			.01			.33[ac]		
	16	.00	.04		-.06	.01		-.06	-.15[ab]		.08	.17[c]	
	19		.02	.12		.01	-.01[a]		.03	.06		-.01	-.03
Shyness	11	-.01			-.01			-.05			-.25		
	16	.00	-.02		.01	-.05		.05	-.15[a]		-.04	-.25	
	19		.02	-.05		-.01	-.03		-.02	-.12		.32[abc]	-.16
Effortful	11	-.08			-.15			-.13			-.01		
Control	16	-.06	-.19		-.10	-.21		-.14	-.10		-.26	-.02	
	19		-.13	-.18		-.17	-.18		-.17	-.12		-.40[a]	-.21

Note. High PDadv and high G were defined as the highest 15%. GxPDadv included individuals high on both G and PDadv. [a] = correlation differs from low sensitivity group, [b] = correlation differs from high G group, [c] = value differs from value in high PDadv group.

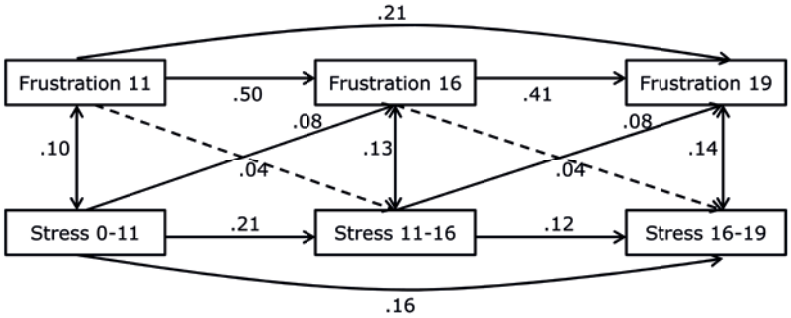

Figure 2. Frustration. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. * = path coefficient (beta) < .05.

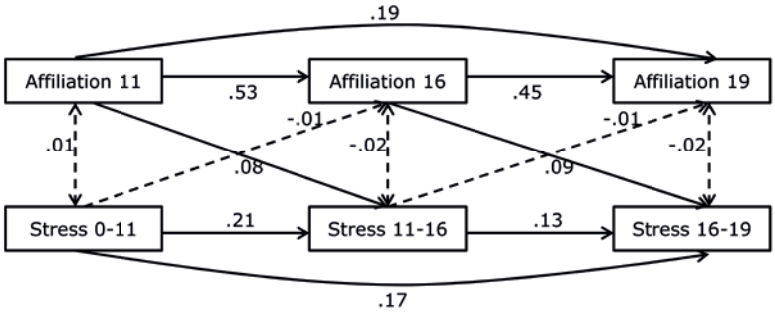

Figure 3. Affiliation. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. * = path coefficient (beta) < .05.

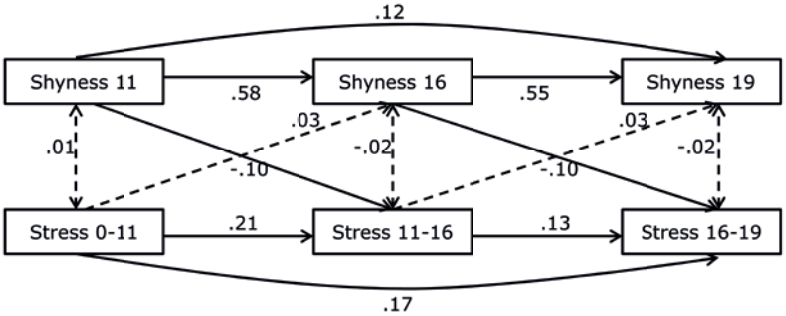

Figure 4. Shyness. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. * = path coefficient (beta) < .05.

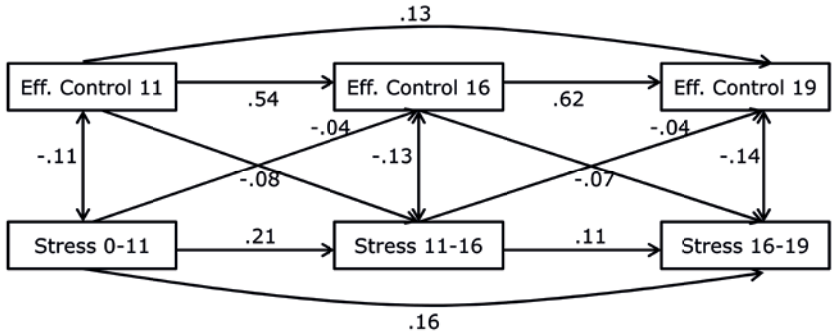

Figure 5. Effortful control. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. * = path coefficient (beta) < .05.

DISCUSSION

This study aimed to elucidate the longitudinal associations between stressful events and temperament during late childhood and adolescence, and to investigate alternative sources of sensitivity to the influence of stressful events on immediate and subsequent temperament. Using three waves of data from a large population cohort we were able to show that for fear and frustration a stress-effect model was most appropriate: stressful life events led to changes in temperament. For affiliation and shyness a trait-effect model was most appropriate: temperament elicited life-events, whereas for effortful control a bidirectional model was most appropriate. Using a cross-lagged modeling approach our study seems to be one of the first disentangling the direction of the associations between stressful events and temperament in more detail.

Additionally, we studied moderation effects by plasticity genes (G), prenatal adversity (PDadv) and the interplay between G and PDadv (GxPDadv) on the effect of stressful events on temperament. Our preliminary analyses suggested that individuals with both high genetic plasticity and PDadv were more sensitive to stressful events during adolescence compared to other individuals. In the multi-group analyses in Mplus only some effects for the high PDadv adolescents turned out to be significant. No significant effects were found of plasticity genes or GxPDadv. Even though genetic plasticity is a popular target for studies on sensitivity, the current approach was new to the literature in two ways: First, we were one of the first using a cumulative plasticity index instead of single plasticity alleles. Second, based on findings from epigenetic studies, we included the prenatal environment as a trigger of the moderating effect of genes on later stress sensitivity.

Stress-effect, trait-effect or bidirectional effects

Our findings provided evidence for different longitudinal associations between stress and various temperament traits. Adolescents who were exposed to more stressful events showed higher subsequent levels of fear and frustration. However, no evidence was found for either

fear or frustration predicting subsequent stressful events, providing support for the, often assumed, stress-effect model. These findings are in line with previous studies on stress and traits related to emotional instability. Our finding is not in line with studies suggesting bidirectional effects between life events and emotional instability (Middeldorp et al., 2008) and depression (Waaktaar, Borge, Fundingsrud, Christie, & Torgersen, 2004). These different findings might be a result of the age of the sample (adults versus adolescents), or of the events under study. For example, Middeldorp and colleagues included illness of self in their analyses, whereas we only included illness of significant others. Thus, the nature of the association between events and emotional instability might differ dependent on the events under study. Negative, stressful events may all affect emotional instability to some extent, because exposure to stressful events may reflect an instable, dangerous environment, in which it can be adaptive to be fearful. In contrast, emotional instability might mainly predict those events that are likely to be a consequence of one's own behaviour.

The opposite pattern was found for affiliation and shyness, the two traits related to the domain of extraversion. Adolescents who were high on affiliation or low on shyness were more likely to be exposed to subsequent stressful events. Stressful events did not significantly predict either subsequent affiliation or subsequent shyness. In our previous study, departing from a stress-effect model, we found modest associations between stressful events and changes in affiliation and shyness (Laceulle et al., 2012). When further qualifying these effects using our current cross-lagged approach, it seems that the longitudinal association between stress and traits related to extraversion, is better accounted for by a trait-effect model than by the stress-effect model. A cross-lagged approach controls for concurrent associations at time 1, correlated change between variables (the within waves paths after time 1) and the stability of both temperament and stressful events. Consequently, a cross-lagged model is more conservative way to study longitudinal associations than the ANOVA's used in our previous two-wave study, and subsequently, results in significant paths only for substantial associations. Some previous studies already showed that extraversion may predispose people to experience normative life events, like starting a romantic relationship (Neyer & Lehnart, 2007; Specht et al., 2011). Although these studies did find support for the influence of extraversion on subsequent exposure to negative life events, it may be that people high on extraversion have both more frequent and more intense interactions with their environment than people that are more reticent, resulting in a larger number of both positive and negative events. Seemingly, life events cannot be viewed as a source of influence on extraversion.

Bidirectional effects were found for effortful control, related to the domain of conscientiousness. Exposure to stressful events predicted lower effortful control and vice versa, although paths from effortful control to subsequent stress tended to be stronger than paths from stressful events to effortful control. Literature on effortful control as a predictor of stressful events is scarce. Negative events have not previously been found as a predictor of subsequent effortful control, except in our own study (Laceulle et al., 2012). Neither Lockenhoff

and colleagues (2009) nor Specht and colleagues (2011) found an effect of stress on changes in conscientiousness. Overall, the findings on the longitudinal associations between stressful events and consciousness are not conclusive. It seems clear that a stress-effect model does not adequately reflect the associations. Future research using a cross-lagged approach may replicate the bidirectional associations found in our study.

Taken together, our hypothesized bidirectional associations for fear and frustration, and trait-effect models for affiliation, shyness and effortful control were only partly confirmed. Although we did not find support for bidirectional associations between stress and either fear or frustration, our findings clearly support the importance of taking into account the direction of the effects. While a stress-effect model may follow naturally from the established associations between stress and psychopathology, and between traits and psychopathology, this model could only account for an effect of stress on fear and frustration. A trait-effect model seemed to reflect the association between stressful events and affiliation and shyness much more adequately. A bidirectional model was found for the association between stress and effortful control.

Sensitivity to stressful events

Plasticity genes. Our preliminary analyses suggested that bivariate correlations between stressful events and temperament were similar in adolescents high on plasticity genes and in adolescents low on plasticity genes and PDadv. Indeed, no moderation effect was found of plasticity genes in the cross-lagged multi-group analyses. It might be that the plasticity genes we included (and were proposed by Belsky and Pluess, 2009) do not modify the impact of stress on temperament at all. Previous studies on plasticity genes tended to focus on the association between stress and psychopathology, not between stress and temperament. Although some studies have suggested that the same genes act on temperament as on psychopathology, it might be that other genes, acting on different physiological systems, play a more important role on the association between stress and temperament than the gens we included. In addition and as previously described in a TRAILS study by Stavrakakis and colleagues (2012), the exact functioning of certain polymorphisms is not entirely clear. For example, even though the TPH1 gene has consistently been reported as a plasticity gene, findings have been mixed on which allele is the plasticity allele (Viikki et al., 2010). It has also been suggested, that it depends on the environmental factor and the outcome under study which allele is the plasticity allele, such as in the COMT gene (Nederhof, Belsky, Ormel, & Oldehinkel, 2012). Another possibility, in line with our hypotheses, is that plasticity genes do influence stress sensitivity only if they are activated. When a high plasticity genes group includes both children in which the alleles are active and children in whom the alleles are inactive, effects will necessarily be weaker and more difficult to detect. This would explain both the non-significant moderation effect of genetic plasticity in the current study and the inconsistent findings of many previous GxE studies.

Prenatal adversity. The bivariate correlations suggested that only a few of the correlations between stressful events and temperament were higher in adolescents high on PDadv than in adolescents low on plasticity genes and PDadv and adolescents high on plasticity genes. Nonetheless, significant effects were found of prenatal adversity alone on the associations between stressful events and frustration. When further examining these effects it turned out that only one or two paths of our model differed significantly between children low/intermediate and children high on PDadv. For the majority of the paths we examined, no significant difference was detected. The lack of a consistent moderation effect of PDadv is in line with our expectation that prenatal anxiety per se does not moderate the longitudinal association between childhood stress and psychological difficulties. Taken together, it seems that, although prenatal adversity can increase the risk for later psychopathology (e.g., O'Connor et al., 2003) early adversity alone does not increase stress sensitivity during childhood and adolescence.

Plasticity genes + PDadv. Given the findings from epigenetic studies, we hypothesized that prenatal adversity plays a key role in the moderating effect of genes on later stress sensitivity. More specific, we expected that the impact of stressful events on subsequent temperament would be substantially stronger in adolescents with both a large number of plasticity genes and high levels of prenatal adversity than in other adolescents. The bivariate correlations suggested a stronger effect of stressful events on fear in adolescents high on GxPDadv, than in adolescents low on both plasticity genes and prenatal adversity and adolescents high on plasticity genes but low on PDadv. Although correlations between stressful events and fear were in magnitude substantially higher in the GxPDadv group (*r* is about .4) compared to the high PDadv but low G group (*r* is about .1), this difference was not statistically significant. The difference between adolescents high on G and PDadv and the other adolescents was not significant in the cross-lagged multi-group analyses either. This might suggest a large effect in a small group (n = 34 for the GxPDadv group), hence, although caution is needed, the present findings warrant interest and interpretation.

First, the strong correlations in the GxPDadv group between stressful events and direct and future fear and (to a smaller extent) frustration, but not so much between stressful events and the other traits, may not be surprising. Although we explored the modifying effect of GxPDadv on the associations between stress and respectively affiliation, shyness (both related to the domain of extraversion) and effortful control (related to conscientiousness), there was no clear rationale to expect this to be the case. In contrast, fear and frustration are both traits related to emotional instability, the domain that has most consistently been found to be affected by stressful events, as these traits reflect behaviours that are adaptive in unstable or dangerous environments. That they also correlate strongest with psychopathology might be another reflection of the consequences of living in an unstable environment (Belsky, Steinberg, & Draper, 1991).

Second, as described in the introduction, the prenatal period has been suggested to be a sensitive period for epigenetics, that is, the (de)activation of genes (e.g., Lillycrop & Burdge,

2010; Oberlander et al., 2008; Weaver et al., 2007). One reason for this sensitivity is that the first period of life may prepare individuals for its future (e.g., Gluckman, Hanson, & Beedle, 2007). If children grow up in an unstable, stressful environment, it might be adaptive to adjust their behaviour in a robust way, that is, through turning on and off certain genes. Although we did not study epigenetics at a molecular level, it seems plausible that the plasticity genes were turned on by early adversity. If an individual was high on plasticity genes, this might have resulted in an increased sensitivity and hence a stronger reaction to stressful events later in life. Consequently, our GxPDadv group may have included more children in whom plasticity genes were turned on and able to affect behaviour, than in our plasticity genes but no PDadv group. As a result, our GxPDadv group was somewhat more sensitive to childhood and adolescent stress. As mentioned before, this line of reasoning does not only explain the results of our current study, but may also help understand the replication problems of classic candidate-gene studies. Classic gene-environment studies usually simply compare environmental effects in individuals with and without a certain plasticity gene. It is likely that of the 'high plasticity' individuals in these studies, a substantial proportion had a plasticity allele that was not active, or turned on. The current study is one of the first selecting a subgroup of high-plasticity individuals in with an increased likelihood that the plasticity alleles are actually turned on.

Some potential weaknesses need to be considered. Again, the size of the group with adolescents high on plasticity genes and PDadv was small. Consequently, the high correlation between stressful events and temperament did not significantly distinguish from the more modest correlations found in the other adolescents in the conservative cross-lagged moderation analyses. Although we used a large population sample, it seems clear that an even larger sample is needed to get an adequate number of individuals high on both early adversity and plasticity genes. Second, in the current study we included a selection of prenatal adversities as well as a selection of childhood and adolescence stressful events based on previous studies in our sample (Laceulle et al., 2012; Nederhof et al., 2010). It might be that including other types of events results in different findings. For example, since the current study only included negative life events, our findings focus on the possible (disease) risk of being high on both plasticity genes and PDadv. In line with the idea of differential susceptibility, it might be that our GxPDadv group is not only more sensitive for future adversity, but also to a positive environment (Pluess et al., 2011).

Another limitation that needs to be mentioned is that we did not take into account (de) activation of genes as a result of the interplay between the genome and the environment during childhood and adolescence. As recently emphasized by Charney and colleagues, the (de)activation of genes is an ongoing process that is not exclusive to the prenatal period (Charney, 2012). Nonetheless, we think that the evidence showing that the prenatal period can be considered a sensitive period for epigenetics (Charney, 2012), as well as for programming effects in general (O'Connor et al., 2003), provides a reasonable rationale to select prenatal adversity as a key component in the moderating role of plasticity genes. Replication using

our design, in combination with the actual measurement of epigenetic processes, could test whether our findings can indeed be explained by activation of (plasticity) genes in the prenatal period. This would substantially add to the current knowledge of the association between genes and later stress sensitivity, as well as to the lack of replicability that seems characteristic for gene-environment studies.

Summary

The results of the present study indicate that although stressful events and temperament traits are associated from late childhood to adolescence, the direction of the effects depends on the temperament trait under study. Whereas the traditional stress-effect model adequately represented the association between stress and fear and between stress and frustration, a trait-effect model may better reflect associations between stress and affiliation and shyness, whereas a bidirectional model may better reflect the association with effortful control, a trait related to conscientiousness. These findings show the importance of taking into account the direction of the effects when studying longitudinal associations between stressful events and temperament. Secondly, correlations between stressful events and temperament were stronger in individuals high on both plasticity genes and prenatal adversity (PDadv) than in other individuals. Although the differences were not statistically significant in the more conservative multi-group analyses, and caution with interpretation is recommended, our findings seem to supports the important role of epigenetics in GxE studies and warrant further interest in future research.

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Stressful events and psychological difficulties: Testing alternative candidates for sensitivity

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ABSTRACT

The current study investigates the longitudinal, reciprocal associations between stressful events and psychological difficulties from early childhood to mid-adolescence. Child age, sex, prenatal maternal anxiety, and difficult temperament were tested as sources of sensitivity, that is, factors that may make children more sensitive to stressful life events. Analyses were based on data from 10,417 children from a prospective, longitudinal study of child development. At ages 4, 7, 9, 11, and 16 years, stressful events and psychological difficulties were measured. Prenatal anxiety was measured at 32 weeks of gestation and difficult temperament was measured at 6 months. Children exposed to stressful events showed significantly increased psychological difficulties at ages 7 and 11 years; there was consistent evidence of a reciprocal pattern: psychological difficulties predicted stressful events at each stage. Analyses also indicated that the associations between stressful events and psychological difficulties were stronger in girls than in boys. We found no evidence for the hypothesis that prenatal anxiety or difficult temperament increased stress sensitivity, that is, moderated the link between life events and psychological difficulties. The findings extend prior work on stress exposure and psychological difficulties and highlight the need for additional research to investigate sources of sensitivity and the mechanisms that might underlie differences in sensitivity to stressful events.

INTRODUCTION

Exposure to stress has been related to a heightened vulnerability to the development of later psychopathology (Brown & Harris, 1978; Kendler, Karkowski, & Prescott, 1999; Ormel, Oldehinkel, & Brilman, 2001). For example, Kendler and colleagues provided evidence that stressful life events have a substantial causal relationship with the onset of episodes of major depression (Brown & Harris, 1978; Kendler et al., 1999; Ormel et al., 2001). However, not everyone exposed to stressful events will develop behavioural or mental problems. In the current paper we examine several possible sources of variation in the link between stressful events and psychological difficulties in a large longitudinal cohort study. In line with the idea that individuals differ in sensitivity to their environment, we test the hypothesis that the association between stressful events and psychological difficulties is moderated by child age, sex, prenatal stress exposure, and difficult temperament.

Stressful life events and children's psychological difficulties

Several theoretical frameworks have proposed alternative mechanisms that may account for the presumed individual differences that moderate the effects of stress on psychological well-being. For example, risk exposures may accumulate and amplify the impact of (subsequent) stress on 'sensitive individuals' (the 'diathesis stress/dual risk' theory (Monroe & Simons, 1991). Recent research has suggested that, in addition to suffering more from an adverse environment, sensitive or susceptible children may also benefit relatively more from a positive environment (the 'differential susceptibility' and 'biological sensitivity to context' theory (Belsky & Pluess, 2009; Boyce & Ellis, 2005; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & Van Ijzendoorn, 2011). For example, using a randomised controlled trial, Scott and O'Connor showed that children who exhibited emotionally-dysregulated behaviour pre-treatment were more responsive to improvements in parental care that were experimentally induced (Scott & O'Connor, 2012). These models imply that individual characteristics can moderate the association between environmental influences and child outcomes, making certain children more sensitive than others, probably for better and certainly for worse. Nonetheless, debate remains about the factors that might moderate the link between stress and well-being and the robustness of this effect.

The developmental process that we focus on in this study is the link between stress and psychological difficulties. This is a natural target for studies of developmental sensitivity because it has a long history in developmental science (Caspi et al., 2003). The current study adds to the existing literature by using five occasions of measurement, from preschool-age to mid-adolescence. Moreover, the longitudinal design allowed us to examine the reciprocal associations between stress exposure and psychological difficulties. Although this has hardly been studied so far, studying reciprocal associations is significant because there is some evidence that depressive symptoms may evoke stressful conditions and events rather than the other way around (Hammen, 1991; Kendler et al., 1999; Waaktaar, Borge, Fundingsrud,

Christie, & Torgersen, 2004). For example, Kendler and colleagues showed that about one-third of the association between stressful events and onsets of depression was non-causal, suggesting that individuals predisposed to major depression select themselves into high-risk environments (Kendler et al., 1999). Consequently, it seems plausible that influences between stress exposure and behavioural and emotional difficulties can also be bidirectional. Accordingly, we will model the reciprocal relations between stressful events and psychological difficulties using a cross-lagged approach.

Factors moderating the link between stressful events and psychological difficulties in children

Several factors have been identified that may moderate the link between stressful life events and psychological difficulties (Grant et al., 2006; Seifer, Sameroff, Baldwin, & Baldwin, 1992). The proposed study adds to the growing literature on individual differences in sensitivity to stress in several ways. First, we adopt a longitudinal design, a feature that has been missing in most studies in this area. Second, we consider several alternative sources of individual sensitivity: age, sex, prenatal maternal anxiety, and temperament.

Age as a source of sensitivity. The extent to which there are developmental changes in an individual's sensitivity to environmental exposures such as stress is a major area of research interest, but questions remain about when in development children may be most sensitive to their environment (O'Connor, 2003). One hypothesis is that early life is a period of greatest sensitivity because the infant brain is most adversely affected by the psychological/biological effects of stress and that these effects are carried forward in development (De Bellis et al., 1999a; Lupien, McEwen, Gunnar, & Heim, 2009; Teicher et al., 2003).

Alternatively, specific brain regions might have their own particular sensitive periods to the effects of stress (Andersen, 2003; Lupien et al., 2009; Teicher et al., 2003); that is, sensitivity may not be a linear, monotonic feature of development. For example, adolescence may also be a sensitive period because of anatomical and neurohormonal changes during these years (Spear, 2000). Indeed, evidence has been found for increased biological sensitivity to stress during adolescence, both in the brain (Perlman, Webster, Herman, Kleinman, & Weickert, 2007) and with regard to physiological stress reactivity (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009) and temperament (Laceulle, Nederhof, Karreman, Ormel, & Van Aken, 2012). Less is known about adolescent-specific sensitivity to stressful events and psychological difficulties. Some evidence has been found for a stronger association in children compared with adolescents (Grant et al., 2006), but findings were inconsistent and only studied in a few cases based on broad age ranges. Thus, our first aim is to investigate the association between stressful events and psychological difficulties and compare the strength of the effects at different stages in childhood and adolescence. Using five waves of data collected longitudinally from early childhood until age 16 we will examine whether or not specifically vulnerable age periods can be distinguished.

Alternative sources of sensitivity. Child characteristics or early exposures might also increase children's sensitivity to stress exposure. *Child sex* is probably the most widely researched moderator in studies on the association between stressful events and problem behaviours (Grant et al., 2006; Oldehinkel & Bouma, 2011). Results are not totally consistent, but there is a suggestion that boys may be more sensitive during early childhood (Van Den Bergh, Mulder, Mennes, & Glover, 2005), whereas girls display more sensitivity during adolescence (Davies & Windle, 1997). Consequently, our second aim is to investigate whether the association between stressful events and psychological difficulties is similar for boys and girls.

Previous studies have shown influences of *maternal prenatal anxiety and stress* on foetal brain development, affecting behavioural, emotional, cognitive development and stress physiology that may underlie psychological symptoms (Glover, O'Connor, & O'Donnell, 2010; Mastorci et al., 2009; Talge, Neal, & Glover, 2007). The developmental programming model that underlies much of this research predicts that prenatal maternal anxiety would heighten sensitivity to future stress. The presumed mechanism is through the programming of stress axes especially the HPA axis, a process that has been reported both in experimental animal work and in human studies (Glover et al., 2010). In the present study we will investigate if prenatal anxiety moderates the effect of environmental influences on psychological difficulties.

Another factor that may account for increased sensitivity to the effect of environmental influences on psychological difficulties is *difficult temperament*. Difficult temperament has been a focus of several studies on sensitivity, in particular from the perspective of the differential susceptibility hypothesis, both in human samples (Seifer et al., 1992; Velderman, Bakermans-Kranenburg, Juffer, & Van Ijzendoorn, 2006) and in rhesus monkeys (Suomi, 1997). These studies suggest that difficult temperament moderates the association between stress exposure and child difficulties. Accordingly, we hypothesized that children with a difficult temperament show a stronger link between stress exposure and psychological difficulties than children without a difficult temperament.

METHODS

Sample

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a longitudinal, prospective study of women, their parents, and an index child. A detailed description of recruitment, dropout, and other methodologies can be found in Golding and colleagues (Golding, Pembrey, & Jones, 2001). For the current study five waves of data on stressful events and psychological difficulties were used. Inclusion criteria for these analyses were that 1) the child was the first born or only child in the family participating in ALSPAC, 2) the gestational age at delivery was at least 32 weeks, 3) weight at birth was at least 1500 grams. This resulted in a sample of 10417 children (68,43 % of the children enrolled in ALSPAC during mothers' pregnancy).

Measures

Psychological difficulties. Psychological difficulties were measured using the Strengths and Difficulties Questionnaire (SDQ). The questionnaire was completed by the mothers for their children at age 4, 7, 9, 11 and 16. The SDQ asks about psychological attributes, some positive and others negative. We used the total difficulties score, based on 20 items on emotional symptoms, conduct problems, hyperactivity/inattention and peer relationship problems. The SDQ's emphasis on strengths as well as difficulties makes it particularly acceptable to community samples. Moreover, because of the limited number of items it has been widely used in epidemiological, developmental and clinical research (Goodman & Scott, 1999). It has well-established consistency and diagnostic predictability. Reliability of the SDQ is good, whether judged by internal consistency (mean α = .73) or cross-informant correlation (mean: 0.34). Internal consistency of the total problem scale was slightly lower in the current sample, ranging from .55-.70 across waves. Also longitudinally, the SDQ has been found to be adequate, with test-retest stability after 4-6 months on average .62 (Goodman, 2001). In the current sample, test re-test stability of the SDQ across the different waves was substantial, although proportionally decreasing with increasing time intervals: Total problems T1-T2 *r* = .58; T1-T3 *r* = .52; T1-T4 *r* = .45; T1-T5 *r* = .43; T2-T3 r = .70; T2-T4 *r* = .64; T2-T5 *r* = .59; T3-T4 *r* = .72; T3-T5 *r* = .66; T4-T5 *r* = .75.

Life events. Stressful events were measured using a questionnaire on life events that may have brought changes to their life and that occurred *since the previous assessment*. Some questionnaires were combined to match the SDQ assessments. This resulted in 5 waves of life event data covering events that occurred from birth-age 4, age 5-7, age 8-9, age 10-11 and age 12-16. All data were obtained from the mother, except data on events that occurred between age 12 and 16 which were obtained from the adolescent. Events were included that 1) were measured at all waves 2) have previously been found to be likely to be experienced as stressful and bring change to someone's life (McMahon, Grant, Compas, Thurm, & Ey, 2003) and 3) are family related events that can be reliably reported by the mother as well as by the adolescent. The 7 events included were illness of a family member, illness of a relative, death of a family member, death of a relative, loss of a job by a parent, problems with the law of one of the parents, and death of a pet (McMahon et al., 2003). Internal consistency of the stress sum scale was modest (ranging from α = .25 at T1, to .34 at T2 and T4), which seems to be in accordance with what could be expected given that the scale consists of relatively independent events. Test re-test correlations of the stress scores across the different waves were modest: T1-T2 *r* = .27; T1-T3 *r* = .22; T1-T4 *r* = .22; T1-T5 *r* = .10; T2-T3 *r* = .25; T2-T4 *r* = .20; T2-T5 *r* = .08; T3-T4 *r* = .35; T3-T5 *r* = .10; T4-T5 *r* = .13.

Prenatal maternal anxiety. Maternal anxiety was measured at 32 weeks in pregnancy using the 16 anxiety items from the Crown-Crisp index, a validated self-rating inventory (Sutherland & Cooper, 1992). In this sample, the internal consistency was .82 (O'Connor, Heron, Golding, Beveridge, & Glover, 2002). We focus on 32 weeks' gestation previously we found a greater effect of prenatal anxiety in late rather than early pregnancy (O'Connor et al., 2002). There is

no well-established clinical cut-off for this measure; we therefore identified as highly anxious those mothers who scored in the top 15%.

Temperament. Temperament was assessed at 6 months with the Carey Infant Temperament Scales (Carey & McDevitt, 1978). The original version, consisting of nine domains, was developed from the work of Thomas and Chess (Thomas & Chess, 1977) on childhood temperament. In our sample the original nine domains were measured, but eleven of the questions were not used in the ALSPAC study because of poor response rate in pilot work; the average internal consistency of the ITQ domains was > .80. We focused on the domain most closely related to 'difficult temperament' as referred to in the literature: mood. The mood scale consisted of nine items rated on a 6-point scale. Parents complete each question using a 6-point scale response, from "almost never" to "almost always". Similar to prenatal anxiety, we identified children as having a difficult temperament using a cut-off at 15%. The scale has demonstrated good test-retest reliability and internal consistency, and normative data exist (Carey & McDevitt, 1978).

Statistical analyses

Changes in psychological difficulties and stressful events as well as main effects of prenatal anxiety, difficult temperament and child sex were analysed using repeated measures ANOVA's. Bidirectional relations between stressful events and psychological difficulties from early childhood into adolescence were studied using path analyses with cross-lagged effects. Analyses were done using the statistical software package software Mplus Version 5 (Muthen & Muthen, 2007). In the model, stability of stressful events and the psychological difficulties over time, and the within-wave correlations included in the model.

First, we examined an (unconstrained) model that included stability paths (both the direct paths and the paths T1-T3, T2-T4 and T3-T5) and within wave correlations of stressful events and psychological difficulties (Model 1). Within-wave correlations refer to Wave 1 cross-sectional correlations and to correlated change in Wave 2, Wave 3 and Wave 4. Then, cross-lagged paths were added to the model and improvement of goodness-of-fit of the model was tested. This was done in three steps. First, we added cross-lagged paths from stressful events to psychological difficulties (Model 2). Second, we added cross-lagged paths from psychological difficulties stressful events without the cross-lagged paths from stressful events to psychological difficulties (Model 3). Third, we examined the bidirectional relations (Model 4). In models 1-4 the paths linking stressful events and psychological difficulties were allowed to vary across age. Then, we compared Model 4 with a model in which all paths were constrained to be equal across waves (Model 5). If Model 5 fitted the data better than Model 4, then this would indicate that the association between stressful events and psychological difficulties was comparable across all ages (i.e., age did not modify this link or act as a differential sensitivity variable). This provided a test of age as a moderator of the link between stressful life events and psychological difficulties. For the model that fitted the data best we reported the model including only the significant paths (Model 6). In exploratory analyses

we also included lag-2 and lag-3 paths, but these additional paths provided no evidence for reliable prediction, and were therefore dropped.

Additionally, multi-group analyses were used to test the three remaining candidates of differential sensitivity: child sex, prenatal maternal anxiety, and difficult temperament. For the developmental model that fitted the data best above, we investigated if concurrent relations and cross-lagged paths varied as a function of 1) high/low maternal anxiety, 2) boys and girls and 3) high/low difficult temperament (Models 7-12).

To determine the goodness-of-fit of the models we used the Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA). CFI should be larger than .90, and the RSMEA smaller than .10. Model comparisons were conducted using Robust χ2 difference tests (Satorra & Bentler, 2001). We selected the most parsimonious model in case of non-significant differences in Robust χ2.

RESULTS

Preliminary analyses

First we compared children with postnatal data (responders, n = 10417) with children who only had data on prenatal anxiety and child sex (non-responders, n = 3114). The comparison showed that the proportion of boys was slightly higher in the non-responders than in the responders (53.2% vs. 51.3%), χ^2 (1) = 3.22, p = .073. The groups did not differ with regard to prenatal anxiety, χ^2 (1) = .67, p = .414. Full information maximum likelihood (FIML) was used to deal with missing data. In the Mplus FIML procedure, individual missing data patterns are assessed, and means and covariances for each missing data pattern are calculated to inform the observed information matrix (Muthen & Muthen, 2007). The observed information matrix is used to generate estimates.

Descriptives

Descriptive statistics are presented in Table 1. As the waves were unequally distributed over time, we reported the absolute number of events that occurred between two assessments and the number of events corrected for the number of months since the previous assessment ((number of events/time gap in months)*100). In the analyses we used the corrected life event variable. Intraclass correlation coefficients for the SDQ total difficulties score across age ranged from .43 to .75 ($p < .001$) and for the life events across age from .08 to .26 ($p < .001$). Bivariate correlations between the different variables under study are reported in Table 2.

Repeated measures ANOVA's showed a significant decrease in psychological difficulties from wave 1 to wave 5 (F (4, 2572) = 219.41, $p < .001$, $\eta^2 = .254$). No association was found between change in difficulties and prenatal anxiety (F (4, 2571) = 1.02, p = .393, η^2=.002) or child sex (F (4, 2571) =1.87, p=.114, η^2=.003). Also the number of stressful events children were exposed to changed over time, although not in a clear linear pattern (Table 1., F (4, 2849) = 1264.26, p <.001, $\eta^2 = .640$).

Table 1. *Means and SD split for sex, prenatal anxiety and temperament.*

	Sex		**Prenatal maternal anxiety**		**Difficult temperament**	
	Boys	**Girls**	**Low**	**High**	**Low**	**High**
Total difficulties	***M* (SD)**	***M* (SD)**	***M* (SD)**	***M* (SD)**	***M* (SD)**	***M* (SD)**
Age 4	9.30(4.68)	8.39(4.40)**	8.56 (4.43)	10.68(4.95)**	8.54(4.41)	10.65(4.55)**
Age 7	7.76(4.91)	6.78(4.46)**	6.99 (4.55)	9.13 (5.31)**	6.96(4.50)	8.99(4.72)**
Age 9	6.82(5.01)	6.04(4.34)**	6.15 (4.51)	8.28 (5.49)**	6.13(4.49)	8.10(4.79)**
Age 11	6.57(4.90)	5.64(4.36)**	5.81 (4.46)	8.01 (5.43)**	5.80(4.46)	7.84(4.58)**
Age 16	6.30(4.46)	5.81(4.34)**	5.79 (4.25)	8.03 (5.07)**	5.80(4.23)	7.84(4.52)**
Stressful events						
Age 0-4 (time gap 47 months)	6.32(2.80)	6.37(2.85)	6.23 (2.80)	7.11 (2.86)**	6.23(2.80)	7.15(2.78)**
Age 4-7 (time gap 34 months)	4.11(3.48)	4.33(3.61)*	4.10 (3.49)	4.90 (3.78)**	4.10(3.50)	4.85(3.66)**
Age 7-9 (time gap 29 months)	6.31(4.45)	6.57(4.45)*	6.31 (4.41)	7.23 (4.59)**	6.34(4.42)	7.06(4.57)**
Age 9-11 (time gap 24 months)	6.40(5.18)	6.94(5.33)**	6.53 (5.21)	7.56 (5.52)**	6.54(5.22)	7.37(5.46)*
Age 11-16 (time gap 64 months)	2.24(1.87)	2.65(1.97)**	2.44 (1.90)	2.78 (2.10)**	2.43(1.92)	2.88(2.05)*

Note. ** Subgroups differ significantly at p < .01, *subgroups differ significantly at p < .05.

Table 2. *Correlation between psychological difficulties, life events, prenatal anxiety, difficult temperament and sex.*

	SDQ age 4	**SDQ age 7**	**SDQ age 9**	**SDQ age 11**	**SDQ age 16**	**Prenatal anxiety**	**Difficult temperament**	**Sex**
Events 0-4	.122****	.137***	.128***	.107***	.118***	.109***	.109****	.009
Events 4-7	.067***	.093***	.091***	.080***	.073***	.078***	.070***	.030*
Events 7-9	.040**	.080***	.080***	.098***	.077***	.070***	.053***	.029*
Events 9-11	.049***	.096**	.087***	.093***	.094***	.068***	.052**	.052***
Events 11-16	.043**	.057***	.114***	.078***	.077***	.058**	.072**	.102***
Prenatal anxiety	.163***	.156***	.155***	.161***	.164***	1.00	.562***	.004
Infant temp.	.158***	.144**	.139***	.143***	.144***		1.00	-.003
Sex	-.100***	-.103***	-.082***	-.100***	-.056**			1.00

Note. *** p < .001. ** p < .01. * p < .05.

Path analyses

First, we compared a model including all stability paths and within-wave correlations with a model including cross-lagged paths from events to difficulties (Table 3, Model 2) and with a model including cross-lagged paths from difficulties to events (Table 3, Model 3). Model fit increased significantly from Model 1 to both models 2 and 3 (Table 3, that is, a significant drop in Chi square). The model fit further improved when bidirectional cross-lagged paths were added (Model 4 versus Model 1, 2 and 3; see Table 3), indicating bi-directional links over time between stressful events and psychological difficulties.

Model fit was significantly worse in Model 5, which constrained all paths to be equal across waves; that is, the associations between stressful life events and psychological difficulties significantly varied as a function of age. Repeated measures findings reported above indicated change in levels of psychological difficulties and numbers of stressful events over time. We deleted all insignificant paths (that is, all dashed lines in Figure 1), and used this model (Model 6) for our further analyses. The fit indexes indicated that the measurement model fit the data adequately (CFI = .988; RMSEA = .031; Table 3).

Next, we tested whether the concurrent associations and cross-lagged paths between stressful events and psychological difficulties were moderated by *prenatal anxiety, child sex and infant difficult temperament*. No moderating effects were found for maternal prenatal anxiety (Model 7 and 8) and difficult temperament (Model 9 and 10), that is, no significant drop in Chi Square. Paths varied significantly between boys and girls, indicating a moderating role of child sex (Model 11 and 12). Model estimates of the cross-lagged paths were stronger in girls than in boys early in life, and some of the paths did not remain significant in boys during middle childhood and adolescence (Figures 2a and 2b).

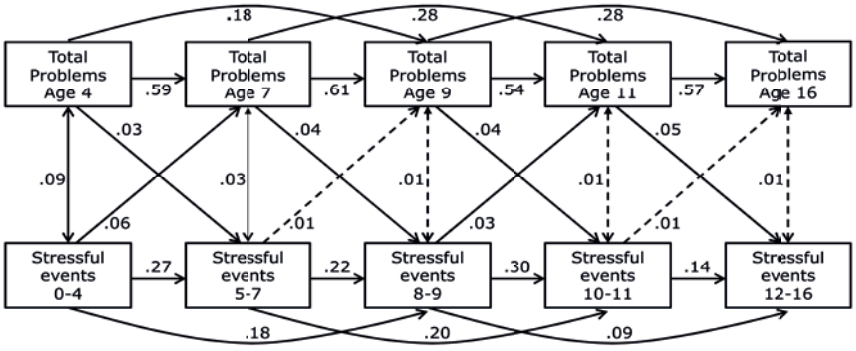

Figure 1. Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. Dashed lines refer to non-significant paths and were deleted from further analyses.

Table 3. *Longitudinal model fit indices and model comparison tests.*

	Model fit indices model comparison tests					Satorra-Bentler Scaled Chi-Square Model comparison tests			
	(df)	**MLr χ2**	**χ2 (*df*)**	**CFI**	**RMSEA**		**TRdχ2**	**Δdf**	***p***
1. Model with stability paths and within wave correlation	26	300.60	11.56	.984	.032				
2. Model 1 + Cross-lagged paths from events to difficulties	22	274.90	12.50	.985	.033	M2 versus M1	25.70	4	<.001
3. Model 1 + Cross-lagged paths from difficulties to events	22	220.83	10.03	.990	.028	3 vs. 1	79.77	4	<.001
4. Model 3 + all cross lagged paths	18	173.49	9.64	.991	.029	4 vs. 1	127.11	8	<.001
						4 vs. 2	101.41	4	<.001
						4 vs. 3	47.34	4	<.001
5. Model 4 fully constrained (all paths fixed across waves)	38	1191.16	31.35	.920	.062	4 vs. 5	1017.67	20	<.001
6. Model 4 only sign. Paths	22	181.62	8.26	.992	.026				
7. Model 4 + prenatal anxiety	66	202.37	3.07	.993	.020				
8. Model 4 + prenatal anxiety constrained	44	206.75	4.70	.992	.027	8 vs. 7	4.38	22	.999
9. Model 4 + diff. temperament	66	172.03	2.60	.988	.040				
10. Model 4 + diff. temp. constrained	44	154.38	3.51	.990	.038	10 vs. 9	17.65	22	.727
11. Model 4 + sex	66	365.07	5.53	.986	.029				
12. Model 4 + sex constrained	44	220.76	5.02	.992	.028	12 vs. 11	144.31	22	<.001

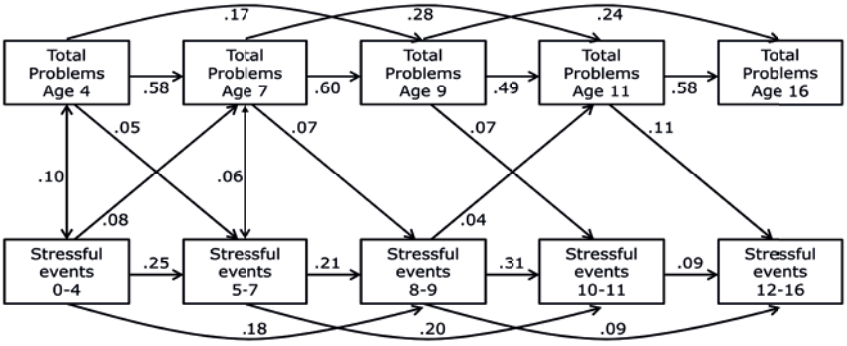

Figure 2a. Girls: Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients.

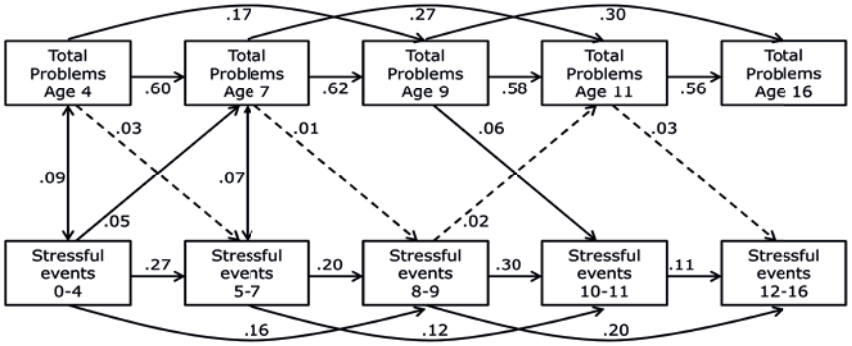

Figure 2b. Boys: Stability paths, within wave correlations and bidirectional cross-lagged paths with beta coefficients. Dashed lines refer to non-significant paths.

DISCUSSION

The current analyses from a large, longitudinal community sample of approximately 10,000 children studies between the ages of 4 and 16 years builds on and extends research on the links between exposure to stressful life events and behavioural problems. Our results indicate that there is increased sensitivity to the effects of stressful life events on psychological difficulties during early childhood and pre-adolescence, and in girls. We found no evidence to suggest that prenatal maternal anxiety or difficult infant temperament moderated the associations between stressful life events and psychological difficulties, cross-sectionally or longitudinally. Furthermore, reciprocal associations were found between stressful events and psychological difficulties from early childhood to adolescence.

Reciprocal associations between stressful events and psychological difficulties

Relations between stressful events and psychological difficulties were bidirectional. Although the literature has traditionally focused on stressful events as a precursor of difficulties, a few

studies suggest that difficulties can also predict subsequent events (Hammen, 1991; Waaktaar et al., 2004). Our results support and extend these findings by demonstrating a dynamic association from early childhood to mid-adolescence. Psychological difficulties, including disruptive and irritable behaviour, predicted the subsequent experience of stressful events that might have been viewed as 'independent' events, or events that were outside of the control of the child. Children's psychological difficulties may have a major impact on the family environment and dynamics, resulting in a complex intertwining of psychological difficulties of the child, and stressful events within the family; this offers further support for the notion that children are active agents in creating their environments. It should be noted that the magnitude of the cross-lagged effects might also be influenced by the fact that stressful events are less stable than psychological difficulties. Taken together, our findings clearly emphasise the need for developmentally-sensitive assessment of how the child is affected by and has a direct role in creating his/her environment.

Factors moderating the link between stressful events and psychological difficulties

The effect of stressful events on psychological difficulties varied across age; in contrast, age did not moderate the link between psychological difficulties and stressful events. This *age-based comparison* assessing the magnitude of association between stressful events and psychological difficulties is new to the literature and indicates that there is no simple, monotonic increase or decrease with age (Andersen, 2003; De Bellis et al., 1999a; Lupien et al., 2009; Spear, 2000; Teicher et al., 2003).

In our study, participants exposed to stressful events showed more psychological difficulties at the ages 7 and 11. At age 9 and 16, participants exposed to stressful events did not show more psychological difficulties. What does explain this inconsistency? First, there is the possibility of false-negative (age 9 and 11) or false-positive (age 7 and 11) findings. Another possibility is that our findings are an indication of age sensitivity. Both human and experimental animal studies have suggested that both children and animals are more sensitive to their environment during some times than others. Early life is a period during which increased sensitivity to stress have been found consistently (De Bellis et al., 1999b). Our findings on age 0-7 are consistent with this. Later in childhood, sensitivity may either gradually decrease, or it might be that adolescence is another period of increased sensitivity, as proposed by Spear and others (Spear, 2000). In both cases it seems plausible that we did not find a significant effect at age 9. With regard to the adolescent years however our findings might imply that 'adolescent sensitivity' mainly applies to the early and not to the late-adolescent years. Alternatively, the measurement gap between early and late adolescence might have been too big to show effects, especially because adolescence has been shown to be a vulnerable period in another study investigating a related outcome (Laceulle et al., 2012) In addition, we note that although paths at specific ages were significant and others were

not, the difference in effect sizes were not substantial. Further studies are needed to confirm early adolescent sensitivity by replication of the current findings as well as by exploring the influence of other possible sources of sensitivity.

Boys and girls were equally sensitive during early childhood, but girls were more sensitive during adolescence (Oldehinkel & Bouma, 2011). The sex difference might be explained by the possibility that boys may be more sensitive to particular events such as violence and poverty (Grant et al., 2006) which were not included in our study. A somewhat related possible explanation is that the events included in the current study occurred within the family environment, and girls may be more sensitive to social/familial stresses than boys (Grant et al., 2006).

Previous studies have found long-term effects of *prenatal maternal anxiety* on children's development (Glover et al., 2010; Hettema, Neale, Myers, Prescott, & Kendler, 2006; Mastorci et al., 2009; Ormel & Schaufeli, 1991; Van Den Bergh et al., 2005). For example, a previous ALSPAC-study showed that children whose mothers experienced high levels of anxiety in late pregnancy exhibited higher rates of psychological difficulties at 81 months of age, providing evidence that prenatal maternal anxiety has a programming effect on the fetus which lasts at least until middle childhood (O'Connor, Heron, Golding, Glover, & the ALSPAC Study Team, 2003). Our study is one of the first human investigations to examine whether or not exposure to prenatal maternal anxiety has a programming effect with regard to future stress sensitivity, that is, whether prenatal maternal anxiety increases children's sensitivity to subsequent stressful events. We found no such evidence, despite a large sample size and notwithstanding the reliable links between prenatal maternal anxiety and children's psychological difficulties that have been found in this sample. This suggests that programming effects of prenatal maternal anxiety are not the same for future psychopathology and for future stress sensitivity. It is not clear if the lack of sensitivity observed here contradicts the findings in rodents (Glover et al., 2010), and what it means for the programming hypothesis that underlies the prenatal anxiety paradigm used in research. Future research is needed to replicate our findings and to further examine programming effects of prenatal maternal anxiety.

Similarly, in contrast to previous research (Seifer et al., 1992), the associations between stressful events and psychological difficulties did not vary according to the child's temperament, as measured with the Carey scales. It is unlikely that our lack of finding might be explained by measurement differences because the Carey scales includes items that have been included in other research, such as irritability, reactivity, and fearfulness. Our failure to detect any interaction might be explained by an alternative measurement factor. Studies reporting temperament to moderate the impact of stress on behavioural outcomes have tended to assess more proximal measures of stress exposure, such as parenting, which may be a more sensitive marker of stress exposure and therefore more likely to show moderation effects.

Our results provide evidence for reciprocal associations between stressful events and psychological difficulties and suggest that this sensitivity might be age-dependent and, to a

more limited degree, sex-dependent. This finding supports the idea that individuals differ in sensitivity to stress exposure, but that there may be a limited number of factors that reliably moderate the impact of stress on behavioural adjustment. In addition, we were limited in our ability to contrast alternative types of interactions, such as the 'diathesis stress/dual risk' and 'differential susceptibility' models (Ellis et al., 2011; Monroe & Simons, 1991). That is because we were limited by having only adverse experiences and did not have robust measures of positive experiences, which is required to differentiate the diathesis stress and differential susceptibility models. Future studies could extend our work by including positive environmental experiences to investigate sensitivity to positive environments as proposed by the differential susceptibility model.

Our study has several strengths, including the number of participants, the longitudinal design and our focus on the reciprocal character of the association between stress and psychological difficulties. Some limitations should be mentioned as well. First, we used the parent version of the SDQ at all ages. The SDQ prediction has been found to work best when SDQs have been completed by all possible informants. However, especially during adolescence, the self report SDQ provides an additional (although not better) source of information, particularly for emotional disorders (Goodman, Simmons, Gatward, & Meltzer, 2000). Future research should include both parent and adolescent ratings from (middle/late) childhood onwards to avoid mono-method bias. Additionally, in the current study we focused on prenatal maternal anxiety as a sensitivity moderator, whereas it seems likely that in particular those children who were exposed to prenatal anxiety were also exposed to some maternal anxiety during childhood. Consequently, SDQ score there may be affected by some respondent bias associated with maternal anxiety at the time of response. Second, the time period between the last two waves was longer than between the other waves. This, in combination with the fact that at age 16 stressful events were reported by the adolescent instead of the mother, may partly explain the drop in number of events exposed to from age 9-11 to 11-16. The large time period may have caused memory bias, and some of the events, e.g., problems with the law of one of the parents, might be somewhat underreported by adolescents. Nonetheless, within a longitudinal population cohort some changes in reporter cannot be avoided. Whereas parental measures may be superior to child measures with regard to child and early adolescent characteristics (including stressful life events), from middle adolescence onwards adolescent reports become increasingly valuable given that adolescents may not share detailed information about certain life events with their parents. Although we only included events in the current study that are likely to be reliably reported both by the parent and by the adolescent, it would be interesting to include both parent and adolescent reports of stressful events in future research. Consequently, the results with regard to the last wave should be interpreted with a caution. Nonetheless, because the association between stressful events and difficulties was not substantially different at this age from other ages, this source of method variance, sometimes inevitable in longitudinal studies across major developmental periods, did not seem to substantially confound study

hypotheses. Also, for the current study we focused on only seven stressful events because of the need to include the same events at all occasions; these events have been identified as stressful, bring change to someone's life (McMahon et al., 2003) and were methodologically feasible to use in a population study spanning 16 years from early childhood to adolescence, but further work is needed to examine more severe and traumatic events. In addition, future research might translate our study to a more experimental design to disentangle causation of our associations in more detail than we could, using a cross-lagged model. Moreover, these studies may included additional potential sensitivity moderators than the factors we included in the current study (e.g., genetic characteristics), in order to test alternative hypotheses. Another limitation is that, because the effects are small as is common in the type of research, the clinical implications are modest.

In conclusion, the longitudinal design allowed us to examine the reciprocal associations between stressful events and psychological difficulties at different ages, and whether evidence could be found for factors accounting for differences in sensitivity. Our results suggest that early childhood and pre-adolescence are sensitive periods to the influence of stressful events, especially for girls. Future research is needed to specify particular mechanisms that may account for why child age and sex moderate the longitudinal links between stressful events and psychological difficulties.

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8

A test of the vulnerability model: temperament and temperament change as predictors of future mental disorders

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ABSTRACT

The current study aimed to test the Vulnerability Model of the relationship between temperament and mental disorders using a large sample of adolescents from the TRacking Adolescents Individual Lives' Survey (TRAILS). The Vulnerability Model argues that particular temperaments can place individuals at risk for the development of mental problems. Importantly, the model may imply that not only baseline temperament predicts mental problems prospectively, but additionally, that changes in temperament predict corresponding changes in risk for mental problems. Data were used from 1195 TRAILS participants. Adolescent temperament was assessed both at age 11 and at age 16. Onset of mental disorders between age 16 and 19 was assessed at age 19, by means of the World Health Organization Composite International Diagnostic Interview (WHO CIDI). Results showed that temperament at age 11 predicted future mental disorders, thereby providing support for the Vulnerability Model. Moreover, temperament change predicted future mental disorders above and beyond the effect of basal temperament. For example, an increase in frustration increased the risk of mental disorders proportionally. The current study confirms, and extends, the Vulnerability Model. Consequences of both temperament and temperament change were general (e.g., changes in frustration predicted both internalizing and externalizing disorders) as well as dimension specific (e.g., changes in fear predicted internalizing but not externalizing disorders). These findings confirm previous studies, which showed that mental disorders have both unique and shared underlying temperamental risk factors.

INTRODUCTION

Temperament and personality have frequently been associated with mental problems. In an overview, Tackett evaluated four models of the association between personality and mental health problems in children and adolescents (Tackett, 2006): 1) The Scar Model, proposing that the development of mental health problems affects personality, 2) The Pathoplasty Model, proposing that personality can affect the manifestation of mental health problems. 3) The Spectrum Model, proposing that personality and mental health problems are manifestations of the same construct and 4) the Vulnerability Model, proposing that personality can place individuals at risk for the development of mental health problems. Until now, the various models all have received some empirical support (De Bolle, Beyers, De Clercq, & De Fruyt, 2012; Kerr, Tremblay, Pagani, & Vitaro, 1997; Klimstra, Akse, Hale III, Raaijmakers, & Meeus, 2010). The models are not mutually exclusive and each of them can explain part of the association between personality and mental problems. Nonetheless, only a few studies have attempted to test one (or more) of the models in detail or tried to elaborate, whereas more direct or sophisticated tests might be needed to elucidate a comprehensive approach to conceptualize the relationship between personality and mental disorders (Tackett, 2006).

In the current study we aim to test the Vulnerability Model using a large sample of adolescents. First, in line with classic tests of the Vulnerability Model, we will examine how baseline temperament at age 11 prospectively predicts *first-onset* mental problems between age 16 and 19. Subsequently and new to the literature, we will examine whether changes in temperament are related to changes in risk for the development of mental problems, above and beyond the effect of baseline temperament. Additionally, the current study will be one of the first assessing mental problems at the clinical level instead of the commonly studied subclinical, or symptom, level. We will use the term mental problems when referring to both clinical and subclinical problems, otherwise we will use respectively symptoms (for subclinical problems) or mental disorders (for clinical problems).

Cross-sectional versus prospective associations

Extensive cross-sectional literature has provided evidence for the association between temperament (often assessed as broader personality traits, like the Big Five) and mental problems, particularly in adults (for meta-analyses see Kotov, Gamez, Schmidt, & Watson, 2010; Malouff, Thorsteinsson, & Schutte, 2005), but also in adolescents. For example, emotional instability has consistently been found to be positively associated with adolescent internalizing symptoms, like depressive symptoms and anxiety (Muris, Meesters, & Blijlevens, 2007). Similarly, using data from the TRAILS sample, evidence was found for associations between internalizing symptoms and both frustration and fear, temperament traits related to the domain of emotional instability (Oldehinkel, Hartman, de Winter, Veenstra, & Ormel, 2004; Oldehinkel, Veenstra, Ormel, De Winter, & Verhulst, 2006). Extraversion was negatively related to adolescent internalizing symptoms (Muris et al., 2007; Oldehinkel et al., 2004; Oldehinkel et

al., 2006). Conscientiousness was negatively related to externalizing symptoms (Lounsbury, Sundstrom, Loveland, & Gibson, 2002; Oldehinkel et al., 2006). Taken together, findings seem to be highly consistent across samples and operationalizations of concepts (adolescents vs. adults; personality vs. temperament questionnaires) and have added substantially to our knowledge about the association between personality and mental problems. Nonetheless, they are not informative with regard to the direction of the effects. Clearly, a longitudinal approach is necessary to test the Vulnerability Model, that is, examine whether traits can place individuals at risk for the development of mental problems. Moreover, to really enable the identification of vulnerability effects, studies should incorporate personality or temperament measures (long) before the onset of mental disorders (De Bolle et al., 2012).

Until now, some longitudinal studies have been published providing support for the Vulnerability Model. In one of the first studies, Huey and Weisz showed that participants high on emotional instability and low on extraversion were more likely to show subsequent internalizing symptoms, whereas those high on extraversion were more likely to show subsequent externalizing symptoms (Huey & Weisz, 1997). Van Leeuwen and colleagues demonstrated that emotional instability and extraversion predicted subsequent internalizing symptoms as well as an association between conscientiousness and externalizing symptoms (Van Leeuwen, Mervielde, Braet, & Bosmans, 2004). However, neither of these studies seem to have controlled for initial symptoms. More sophisticated designs were used by Ormel and colleagues (2005) and Klimstra and colleagues (2010). Using data from the TRAILS sample, Ormel and colleagues (2005) predicted adolescent internalizing and externalizing symptoms from early adolescent temperament and familial loading (parental lifetime psychopathology). Results showed that higher levels of fear and frustration and lower levels of effortful control at age 11 were related to more symptoms a few years later. Klimstra (2010) reported similar associations as in earlier studies, as well as paths from symptoms to personality, suggesting an interplay between personality and symptoms. Additionally, consistent evidence has been provided that the associations (both cross-sectional and longitudinal) between temperament and mental disorders are invariant across sex, despite sex differences in temperament and mental disorders (e.g., Feingold, 1994; Ormel et al., 2005; Verhulst, van der Ende, Ferdinant, & Kasius, 1997).

Temperament change and future mental problems

So far, almost all studies on temperament (or personality) and mental health problems have approached traits as stable features of individual differences. Indeed, longitudinal studies in children and adolescents have provided evidence for substantial stability of traits (McCrae et al., 2000). Nonetheless, particularly over the last decades, an increasing emphasis has emerged on the notion that traits are not developmentally static (McCrae et al., 2000). Similar evidence has come from behavioural genetic studies, providing support for a substantial state (or change) component, in addition to stability (Kandler et al., 2010; Laceulle, Ormel, Aggen, Neale, & Kendler, In press). Most studies on stability and change have focused on

describing change over the life course (e.g., Roberts, Walton, & Viechtbauer, 2006, in a meta-analysis), suggesting that in particular adolescence might be a period of major changes in temperament (e.g., Klimstra, Hale III, Raaijmakers, Branje, & Meeus, 2009). Adolescent temperament change has been found to be often (although not always) in the direction of maturation (for a review see Caspi, Roberts, & Shiner, 2005). Indeed, using data from the TRAILS sample, we previously demonstrated decreases in fear, frustration (related to emotional instability), effortful control (related to conscientiousness), affiliation and shyness (somewhat related to extraversion). Increases were found with regard to high intensity pleasure (related to extraversion, Oldehinkel et al., 2006). In addition to mean level changes, some studies have examined sources of change, either in terms of intrinsic maturational factors (Roberts, Wood, & Smith, 2005) or in terms of environmental factors, such as stressful life events (Laceulle, Nederhof, Karreman, Ormel, & Van Aken, 2012; Löckenhoff, Terracciano, Patriciu, Eaton, & Costa, 2009; Vaidya, Gray, Haig, & Watson, 2002). Little is known, however, on whether inter-individual variation in temperament change is predictive of future mental problems. That is, temperament change may predict corresponding changes in risk. So far, only a few studies have attempted to address personality change and mental problems in adolescents (Akse, Hale III, Engels, Raaijmakers, & Meeus, 2007; De Bolle et al., 2012; Johnson, Hicks, McGue, & Iacono, 2007) although more studies have been performed in adults (e.g., Warner et al., 2004). For example, Akse and colleagues (Akse et al., 2007) used a person-centered approach to demonstrate that adolescents who changed from a more introverted to a more extraverted personality type, showed decreasing levels of internalizing symptoms, while increasing levels of these symptoms were displayed by adolescents who showed the opposite pattern of personality type change. However, changes in personality and changes in symptoms were assessed concurrently. In the current study we will predict the onset of mental disorders between ages 16 and 19 prospectively from baseline temperament at age 11 and as well as from changes in temperament between ages 11 to 16. Given the assumed adaptive value of temperament maturation, it seems plausible that particularly temperament change reflecting the opposite of maturation will be predictive of changes in the risk of being diagnosed. Nonetheless, it might be that also extreme maturation (e.g., major increases in effortful control) are predictive of mental disorders. Therefore, non-linear associations between temperament change and future mental disorders will be explored.

Until now, mental health problems have been operationalized in terms of continuous scores on questionnaires in the majority of studies on temperament and adolescent mental health.. Longitudinal associations between temperament and clinical diagnoses were predominantly investigated in studies with adults. For example, Warner and colleagues examined cross-lagged associations between personality traits and personality disorders in adults (Warner et al., 2004). The current study extends this work by looking at associations between (changes in) temperament and diagnoses from a clinical interview in adolescents. More specific, we will predict first-onset, mental disorders that were diagnosed between age 16 and 19.

Current study

In summary, the main aim of the current study is to investigate whether changes in temperament predict corresponding changes in risk for mental problems. To model our associations prospectively, we ass essed temperament change between age 11 and 16, and included only those psychiatric diagnoses with a first onset between age 16 and 19 (i.e., no diagnoses with an onset before age 16 or recurrent diagnoses).

First, with respect to initial temperament, fear, frustration and effortful control at age 11 are expected to predict mental disorders with an onset between age 16 and 19 (Ormel et al., 2005). Second, we hypothesize that temperament change deviating from normative change (in particular change reflecting the opposite of maturation, Laceulle et al., 2012) can make adolescents more vulnerable to the development of mental disorders a few years later. More specifically, increases in fear and frustration (instead of the normative decreases previously reported), and substantial increases and/or decreases in effortful control (instead of the minor normative decreases) between age 11 and 16 are hypothesized to predict first-onset internalizing mental disorders between age 16 and 19. Additionally, we hypothesize that substantial decreases in affiliations as well as substantial increases in shyness predict internalizing disorders. Increases in frustration, substantial decreases in effortful control and possibly also substantial increases in high intensity pleasure (instead of the modest normative increases) between age 11 and 16 are expected to predict externalizing mental disorders between age 16 and 19. Non-linear associations and sex differences will be explored, as well as associations between (changes in) temperament and specific mental disorders. Given the low occurrence of most specific disorders, we will focus on linear associations between temperament traits and disorders that were diagnosed in more than 10 adolescents.

METHODS

Sample

Data from the first, third and the fourth wave of the TRacking Adolescents' Individual Lives Survey (TRAILS) were used (Ormel et al., 2012). TRAILS is a large prospective cohort study of 2,230 Dutch adolescents, who are followed bi- or triennially from 11 to at least 25 years of age. Sample selection involved two steps. First, five municipalities in the North of The Netherlands, including both urban and rural areas, were requested to give names and addresses of all inhabitants born between in 1990 and 1991, yielding 3,483 names. Simultaneously, primary schools within these municipalities were approached with the request to participate in TRAILS. Of the 135 primary schools, 90.4% accommodating 90.3% of the children, agreed to participate in the study. If schools agreed to participate, parents received information about the study. Shortly thereafter, a TRAILS interviewer contacted parents by telephone to ask whether they and their son or daughter were willing to participate in the study. If both parents and children agreed to participate, parental written informed consent was obtained

after the procedures had been fully explained. Of all children approached for enrolment in the study 76.0% (n= 2,230, mean age = 11.09, *SD* = 0.56, 50.8% girls) were enrolled in the study. Responders and non-responders did not differ with respect to the prevalence of teacher-rated problem behaviour. Furthermore, no differences between responders and non-responders were found regarding associations between socio-demographic variables and mental health outcomes (De Winter et al., 2005). The present study involves data from the first, third and fourth assessment wave. Mean age was 10.5 (SD = .58) at the first wave, 16.1 (SD = 0.59) at the third wave and 19.1 (SD 0.60) years at the fourth wave. The survey was approved by the national ethical committee.

Measures

Temperament. Adolescent temperament was assessed both at age 11 and at age 16 by means of the short form of the parent version of the Early Adolescent Temperament Questionnaire-Revised (EATQ-R, Hartman, 2000; Putnam, Ellis, & Rothbart, 2001). The following six scales were distinguished: Fear (α = .63), Frustration (α = .74), Shyness (α = .84), Effortful Control (α = .86), Affiliation (α = .66) and High Intensity Pleasure (α = .77). Missing items were imputed by means of Corrected Item Mean imputation (CIM; Huisman, 2000).

Temperament scores were re-coded into Reliable Change scores (RC-scores; Jacobson & Truax, 1991). RC-scores are difference scores which take unreliability of measurement explicitly into account (RC-score = $(X_2 - X_1)/S_{diff}$, in which X_1 and X_2 are the scores on the EATQ-scales at age 11 and 16 and S_{diff} is the standard error of the difference between scores at age 11 and 16; Christensen & Mendoza, 1986), thereby making separation possible between true changes in temperament and changes due to measurement error. This explicit correction for measurement error makes RC-scores preferable to more common techniques.

Mental disorders. Presence of mental disorders was assessed during the fourth assessment wave, by means of the World Health Organization Composite International Diagnostic Interview (WHO CIDI), version 3.0. The WHO CIDI is a structured diagnostic interview which yields lifetime diagnoses and age of first onset of each diagnosis according to the definitions and criteria of the Diagnostic and Statistical Manual of Mental disorders (DSM-IV; American Psychiatric Association, 2000). The CIDI has been used in a large number of surveys worldwide (Kessler & Ustün, 2004) and has been shown to have good concordance with clinical diagnoses (Kessler et al., 2009). All TRAILS T4 respondents were invited for the diagnostic interview, of which 84.2% (n = 1584) agreed to do so. Participants were categorized as having no versus at least one first onset between TRAILS T3 (age 16) and T4 (age 19). Adolescents who were categorized as having no first onset disorder between age 16 and 19 included both adolescents who were never diagnosed with a disorder (n = 900) and adolescents with a disorder diagnosed before age 16 (n = 125). With regard to adolescents who were categorized as having a first onset disorder between age 16 and 19 (n = 170), we distinguished between internalizing disorders (total n = 102, including Adult Separation Anxiety Disorder, n = 13;

Agoraphobia, n = 4; Dysthymia, n = 9; Generalized Anxiety Disorder, n = 18; Major Depressive Disorder, n = 93; Panic Disorder, n = 11; Separation Anxiety Disorder, n = 7; Social Phobia, n = 10; Specific Phobia, n = 6) and externalizing disorders (total n = 85, including Alcohol Dependence, n = 42; Conduct Disorder, n = 11; Drug Dependence, n = 47; Oppositional Defiant Disorder, n = 14; Pathological Gambling n = 5).

Statistical analyses

All analyses were performed on complete cases (N = 1195). Associations between temperament change and mental disorders were assessed by means of two sets (internalizing, externalizing) of six logistic regression analyses, one for each temperament trait. In each logistic regression analysis independent variables were included in three steps. Step 1: sex. Step 2: the temperament trait at baseline (age 11) and temperament change between age 11 and 16. Step 3: the interaction between sex and temperament change, to investigate possible moderation of sex, and a quadratic temperament change score, to investigate possible non-linear effects.

Subsequently, additional logistic regression analyses were performed to explore the associations between temperament and the individual disorders. Given the low frequency of some of the individual disorders we limited our analyses to 1) step 2 of the regression analyses, that is, we did not include any of the interaction terms and 2) those disorders that were diagnosed in at least 10 adolescents. This resulted in 9 additional analyses including Adult Separation Anxiety Disorder, Generalized Anxiety Disorder, Major Depressive Disorder, Panic Disorder, Social Phobia, Alcohol Dependence, Conduct Disorder, Drug Dependence, Oppositional Defiant Disorder.

RESULTS

We compared whether the current sample differed from the larger TRAILS sample on any of the study. More girls were included in this sample, compared to the larger TRAILS sample (i.e., 54.5% compared to 50.8%, $Chi^2 = 14.21$, $p < .001$). Also, adolescents in this study were lower on effortful control and higher on fear as measured at T1 (respectively $t = -5.43$, $p < .001$ and $t = 2.5$, $p = .012$). Descriptive statistics for the temperament measures, mental disorders and sex are reported in Table 1. Of the 1195 adolescents, 170 adolescents were diagnosed with a disorder. 85 adolescents were diagnosed with only an internalizing disorder, 68 with only an externalizing disorder and 17 with both an internalizing and an externalizing disorder. Correlations between all study variables are reported in Table S1.

Table 1. *Descriptive statistics.*

		N	Min	Max	Mean	SD
Temperament	Fear	1195	1.00	4.80	3.89	.56
age 11	Frustration	1195	1.00	4.80	2.77	.65
	Affiliation	1195	1.50	5.00	3.89	.56
	High intensity pleasure	1195	1.00	5.00	3.29	.92
	Shyness	1195	1.00	5.00	3.23	.67
	Effortful Control	1195	1.09	5.00	3.29	.69
Temperament	Fear	1195	1.00	4.40	1.94	.64
age 16	Frustration	1195	1.00	5.00	2.69	.69
	Affiliation	1195	1.33	5.00	3.70	.63
	High intensity pleasure	1195	1.00	5.00	3.49	.82
	Shyness	1195	1.00	5.00	2.33	.91
	Effortful Control	1195	1.27	5.00	3.23	.67
Temperament	Fear	1195	-4.26	3.12	-.63	1.02
change 11-16	Frustration	1195	-4.11	2.94	-.12	.97
	Affiliation	1995	-3.95	3.10	-.32	.99
	High intensity pleasure	1995	-3.36	3.16	.24	.94
	Shyness	1995	-3.98	4.28	-.21	1.00
	Effortful Control	1995	-3.87	3,72	-.10	1.02
INT disorders	Yes	102				
	No	1093				
EXT disorders	Yes	85				
	No	1110				
Sex	Boys	544				
	Girls	651				

Internalizing mental disorders

Step 1 of the logistic regression analysis revealed that girls were more likely to be diagnosed with a new internalizing disorder than boys (Exp(B) = .54, B = -.61(1), p = .006). Results of step 2 of the regression analyses are reported in Table 2. Adolescents who, at baseline, were high on fear or frustration, or lower on effortful control, were more likely to be diagnosed with a new internalizing disorder between the ages of 16 and 19 than other adolescents. In addition, the smaller the decreases in fear adolescents showed between age 11 and 16, the more likely they were to be newly diagnosed between the ages of 16 and 19. Similarly, adolescents who showed increases in frustration instead of the normative decreases, had a heightened risk to be diagnosed with an internalizing disorder. Affiliation, high intensity pleasure and shyness were not significantly related to new internalizing disorders. For effortful control,

only the baseline level was associated with new internalizing disorders. Results for the effects of temperament change are depicted in Figure 1. For reasons of clarity we have presented reliable temperament change scores, split for adolescents that were and were not diagnosed with a mental disorder between age 16 and 19. Additionally, neither the interaction between sex and temperament change, nor the quadratic temperament change score (step 3 of the regression analysis) was significantly related to new internalizing disorders.

Table 2. *Temperament change and internalizing disorders.*

		B	S.E.	Wald	df	p	Exp(B)
Fear	Fear T1	.42	.18	5.54	1	.019	1.52
	RC-score	.26	.12	4.46	1	.035	1.29
Frustration	Frus T1	.49	.18	7.35	1	.007	1.63
	RC-score	.46	.12	14.32	1	.000	1.58
Affiliation	Aff T1	.09	.22	.17	1	.684	1.09
	RC-score	-.04	.12	.10	1	.752	.96
High Intensity	HIP T1	-.08	.14	.33	1	.568	.93
Pleasure	RC-score	.04	.13	.08	1	.773	1.04
Shyness	Shy T1	.03	.13	.06	1	.804	1.03
	RC-score	.07	.11	.36	1	.547	1.07
Effortful	Eff C T1	-.48	.18	6.91	1	.009	.62
Control	RC score	-.22	.12	3.82	1	.069	.80

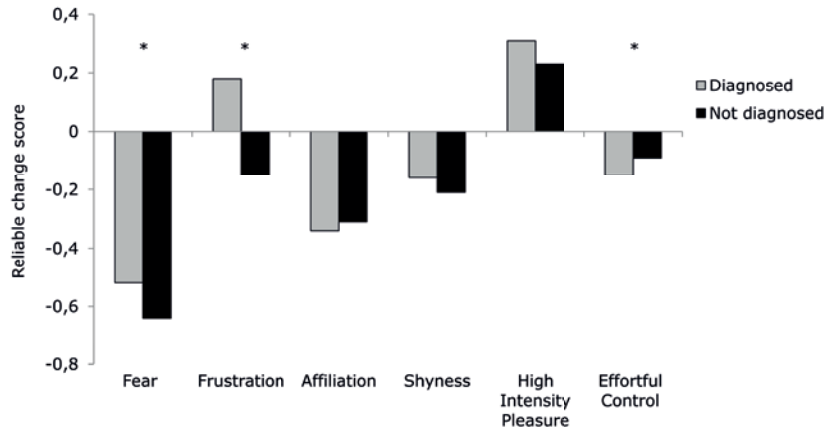

Figure 1. Associations between temperament change between age 11 and 16 and internalizing mental disorders. * = difference between diagnosed and not diagnosed significant at $p < .05$.

Additional analyses revealed that, in general, the same temperament traits (i.e., fear, frustration and effortful control) were related to the individual internalizing disorders in the same direction as to total internalizing disorders. The associations between temperament and internalizing disorders were most consistent for *Major Depressive Disorder*. Both fear and effortful control at age 11 predicted Major Depressive Disorder (respectively ExpB = 1.60, p = .026 and ExpB = .66, p = .053). Effects of changes in these traits were borderline significant (respectively ExpB = 1.30 p = .070 and ExpB = .78, p = .087). Changes in, but not baseline, frustration were also significantly related to Major Depressive Disorder (ExpB = 1.47, p = .007).

Associations between temperament and the anxiety disorders were less consistent. Changes in, but not baseline, fear (ExpB = 1.98 p = .031) and frustration ExpB = 2.64, p = .004) were related to *Adult Separation Anxiety Disorder*. Effortful control was not significantly related to Adult Separation Anxiety Disorder. Frustration at age 11, but none of the other traits nor changes in traits, predicted subsequent *Panic Disorder* (ExpB = 10.27, p = .027). *Generalized Anxiety Disorder* and *Social Phobia* were not significantly predicted by any of the (changes in) temperament traits. Similar to the findings on total internalizing disorders, (changes in) affiliation, surgency and shyness were not significantly related to the individual internalizing disorders (except for Social Phobia, which was significantly predicted by changes in affiliation, ExpB = 2.54, p = .052). Statistics for the non-significant associations are available upon request.

Externalizing mental disorders

Step 1 of the logistic regression analysis revealed that boys were more likely to be diagnosed with a new externalizing disorder than girls (Exp(B) = 1.68, B = .53(1), p = .021). Results of step 2 of the regression analyses are reported in Table 3. Adolescents who were, at baseline, high on frustration or low on effortful control, were more likely to be diagnosed with a new externalizing disorder between the ages of 16 and 19 than other adolescents. In addition, adolescents who increased in frustration between age 11 and 16 were more likely to be diagnosed with an externalizing disorder than adolescents who showed (the normative) decreases. Similarly, the larger the decreases adolescents showed in effortful control between age 11 and 16, the more likely they were to be newly diagnosed between the ages of 16 and 19. Fear, affiliation, high intensity pleasure and shyness were not related to new externalizing disorders, neither in terms of baseline levels nor in terms of change. Results are depicted in Figure 2. Again, neither the interaction between sex and temperament change nor the quadratic temperament change score (step 3 of the regression analysis) was significantly related to new externalizing disorders.

Additional analyses revealed that associations between (changes in) temperament and individual externalizing disorders were highly consistent to those reported for total externalizing disorders. Again, only frustration and effortful control were related to individual externalizing disorders. Frustration and effortful control at age 11, as well as changes in frustration and effortful control between age 11 and 16, predicted *Conduct Disorder* (respectively ExpB = 4.38, p = .025; ExpB = .16, p = .006; ExpB = 3.48, p = .004; ExpB = .37, p = .033). Similarly, frustration

and effortful control at age 11, as well as changes in frustration and effortful control between age 11 and 16, predicted *Oppositional Defiant Disorder* (respectively ExpB = 3.55, $p = .051$; ExpB = 37, $p = .038$; ExpB = 1.84, $p = .051$; ExpB .41, $p = .008$). *Drugs Dependence* was significantly predicted by baseline effortful control (but not frustration) and by changes in both frustration and effortful control (respectively ExpB = .33, $p < .000$; ExpB = 1.48, $p = .042$; ExpB = .44, $p < .001$). Finally, *Alcohol Dependence* was only borderline significantly predicted by changes in frustration and effortful control (respectively ExpB = 1.43, $p = .061$; ExpB = .69, $p = .061$). Similar to the findings on total externalizing disorders, (changes in) affiliation, surgency and shyness were not significantly related to the individual externalizing disorders.

Table 3. *Temperament change and externalizing disorders.*

		B	S.E.	Wald	df	p	Exp(B)
Fear	Fear T1	.15	.20	.54	1	.462	1.16
	RC-score	.20	.14	2.22	1	.136	1.22
Frustration	Frus T1	.65	.19	11.44	1	.001	1.92
	RC-score	.48	.13	13.63	1	.000	1.62
Affiliation	Aff T1	.15	.23	.42	1	.517	1.16
	RC-score	-.09	.12	.62	1	.432	.907
High Intensity	HIP T1	.27	.15	2.97	1	.085	1.30
Pleasure	RC-score	-.15	.15	1.10	1	.294	.86
Shyness	Shy T1	-.06	.15	.15	1	.701	.95
	RC-score	-.02	.13	.03	1	.870	.98
Effortful	Eff C T1	-.86	.20	19.15	1	.000	.42
Control	RC score	-.70	.14	25.04	1	.000	.50

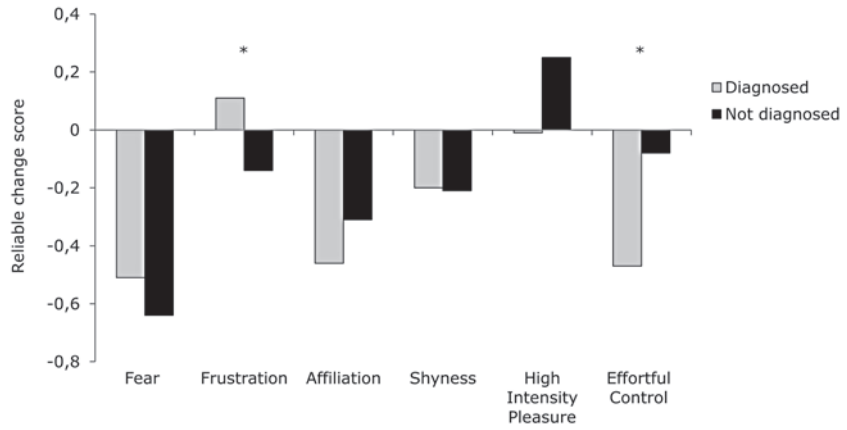

Figure 2. Associations between temperament change between age 11 and 16 and externalizing mental disorders. * = difference between diagnosed and not diagnosed significant at $p < .05$.

DISCUSSION

The current study aimed to test the Vulnerability Model, postulating that personality can place individuals at risk for the development of mental problems (Tackett, 2006). In line with previous studies, associations were found between basal temperament (at age 11) and mental problems a few years later (Muris et al., 2007; Ormel et al., 2005). In addition, and new to the literature, analyses revealed that changes in temperament between age 11 and 16 predicted both internalizing and externalizing disorders between age 16 and 19. The associations that were found between basal temperament and mental disorders provide support for the Vulnerability Model. Most importantly, the results revealed that temperament change has an effect, above and beyond basal temperament. For example, a decrease in frustration proportionally decreased the risk of mental disorders.

Additionally, we tested whether the associations for the groups of internalizing and externalizing disorders also hold for individual disorders. Overall, although the same temperament traits (fear, frustration and effortful control) were related to individual internalizing disorders as to total internalizing disorders in general, the associations between temperament and internalizing disorders seemed to be mainly the result of a strong association between (changes in) temperament and Major Depressive Disorder (MDD). Associations with the various anxiety disorders were less consistent. It might be that age confounds the associations for anxiety disorders. Whereas depression tends to develop during middle adolescence, anxiety disorders often develop earlier. Consequently, adolescents with an anxiety disorder may have been diagnosed already before the age of 16. Disorders with an earlier onset were beyond the scope of the present study. Future research is needed to examine this in more detail.

Interestingly, associations between (changes in) temperament and individual externalizing disorders were much more consistent. Baseline frustration and effortful control as well as changes were related to the individual externalizing disorders. Moreover, the majority of the associations was significant, despite low frequencies, and all associations were in the same direction as found for total externalizing disorders. Seemingly, (changes in) frustration and effortful control are rather general predictors of conduct, oppositional and substance abuse disorders.

Overall, the findings on the internalizing and externalizing disorder variables, as well as on the individual disorders, strengthen traditional tests of the Vulnerability Model, predicting mental problems from a single temperament measure. Moreover, our results provide insight in the long term consequences of temperament *change* on adolescent mental health. Extensive literature has described stability and change of temperament (Caspi et al., 2005), but until now, studies on consequences of change were lacking. Akse and colleagues (2007) reported how changes in personality type were related to changes in anxiety. Also Klimstra and colleagues investigated personality and symptoms longitudinally, but although they modelled associations sophisticatedly, they did not address the question how changes in

traits predicted future problems (Klimstra et al., 2010). Given that we are (one of) the first explicitly investigating the consequences of temperament change, replication is of course in order.

Future research may further investigate possible differences in the nature of the association between baseline temperament and mental disorders on the one hand, and temperament change and mental disorders on the other hand. For example, it might be that baseline temperament is primarily related to more chronic, life-course persistent disorders, whereas temperament change may be related to adolescence-limited disorders (due to a lack of, or delayed, temperament maturation).

Notably, only temperament change that reflected the opposite of maturation (e.g., increases instead of the normative decreases in frustration and larger decreases in effortful control than normative) was predictive of changes in the risk of mental disorders. Although we hypothesized linear associations, we also explored non-linear associations. We did not find evidence for any non-linear association, which seems to suggest that adolescent temperament is unlikely to mature too much to be adaptive. Despite the well established differences between boys and girls regarding both temperament and mental problems, we found no gender differences in the associations between temperament change and psychiatric diagnoses. This is well in line with previous studies on temperament and internalizing and externalizing symptoms, both within TRAILS and in other samples (Klimstra et al., 2010; Ormel et al., 2005).

Our findings may provide support for specificity of etiologies of internalizing and externalizing disorders, as well as for a common pathway. Confirming the Specificity Hypothesis (Kendler, Prescott, Myers, & Neale, 2003), fear at age 11 as well as changes in fear between age 11 and 16 predicted internalizing, but not externalizing disorders a few years later. And, although basal levels of effortful control were related to both internalizing and externalizing disorders, changes in effortful control significantly predicted externalizing, but not internalizing disorders later. Basal levels of frustration, as well as changes in frustration were significantly related to both internalizing and externalizing disorders, corroborating the notion that different types of mental disorders also share underlying risk factors (Neeleman, Bijl, & Ormel, 2004; Ormel et al., 2005). It should be noted, that further research is needed to be conclusive on specificity. Although, for example, fear significantly predicted internalizing but not externalizing disorders, to limit the number of our analyses we did not perform further post-hoc tests to examine whether the relation of fear to internalizing disorders differed significantly from the relation of fear to externalizing disorders.

Our study seems to be the first to prospectively test the Vulnerability Model using mental disorders at the clinical level. Several studies have emphasized the need for replication using a diagnostic interview (Gjerde, Block, & Block, 1988; Klimstra et al., 2010). Our findings confirm findings in the existing literature on symptom level. Other strengths of the current study are the prospective design, that we only included first-time diagnoses with an onset after age 16

(thus, neither diagnoses with an onset before age 16, nor recurrent episodes) and the large sample size (enabling us to study mental problems at the clinical instead of the symptom level).

Nonetheless, the current findings should also be interpreted in the light of some limitations. First, as mentioned before, some of our findings may not only fit the Vulnerability model, but also other explanatory models like the Spectrum model (Tackett, 2006) and the Precursor model (Klein, Kotov, & Buffered, 2011). Like the Spectrum model, the Precursor model posits that temperament and psychopathology are caused by similar etiologic factors. The Precursor model differs from other models in that it assumes a particular developmental sequence, with the temperament traits being evident prior to the onset of the disorder. In the current study we were not able to disentangle the Vulnerability model from either the Spectrum or the Precursor model (or both).

A second issue we did not account for in our analyses is comorbidity between internalizing and externalizing disorders. Previous studies provided consistent evidence for co-occurrence of mental problems at the symptom level. Seemingly, with regard to disorders at the clinical level co-occurrence is less common, only 10% of the adolescents with an internalizing disorder was also diagnosed with an externalizing disorder. Related to this are the issues of interaction and overlap between temperament traits. Given the already large number of analyses, it was beyond the scope of the current study to investigate interactions or to take into account overlap between temperament dimensions. Nonetheless, this clearly would be a valuable addition which could further disentangle unique and shared effects of (changes in) temperament traits.

Third, it may be that temperament changes reflect the development of prodromal symptoms of internalizing and externalizing disorders. Fourth, traits were assessed using a temperament questionnaire and not with the more frequently used Big Three or Big Five. This may give rise to questions regarding the generalizability of our findings. However, the EATQ-R has been suggested to be compatible with measures such as the Big Five (Muris et al., 2007). More general, over the years an increasing emphasis has emerged on the connection between personality and temperament, suggesting that the concepts are largely equivalent and the terms may even be used interchangeably (Klein et al., 2011). Moreover, the EATQ-R has explicitly been developed for early adolescents, making this questionnaire the most suitable for our sample. Additionally, in contrast to the Big Five scales it allows differentiating between different subscales of broader temperament traits. Our finding that frustration, but not fear, both related to emotional instability, predicted externalizing disorders, suggests that the Big Five might be too general to answer our current research question. Unfortunately, the EATQ-R does not include items or scales on positive emotionality/affect, an aspect of extraversion included in most personality questionnaires. Future studies may examine whether the associations between (changes in) positive affect and disorders are similar (that is, non-significant) to high intensity pleasures and affiliation.

To conclude, the current study provided strong support for the Vulnerability Model (Tackett, 2006). Most important, we demonstrated that, in addition to the effect of basal temperament, temperament change can affect the risk for mental disorders a few years later. More specifically, our findings suggested that changes in the opposite direction of maturation increase the risk of mental disorders. This finding confirms and strengthens the traditional Vulnerability Model. Additionally, our findings show that previous work on temperament and internalizing and externalizing symptoms is generalizable to internalizing and externalizing disorders. Finally, consequences of temperament change were both general (i.e., frustration) and dimension specific (i.e., fear), confirming previous studies showing that mental disorders have both unique and shared underlying risk factors (Kendler et al., 2003; Neeleman et al., 2004; Ormel et al., 2005).

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SUPPLEMENTARY MATERIAL

Table S1. *Correlations between the temperament and disorder variables.*

	Temperament age 11						Temperament age 16						INT						EXT				
	Fear	**Fru**	**Aff**	**Hip**	**Shy**	**EffC**	**Fear**	**Fru**	**Aff**	**Hip**	**Shy**	**EffC**	**Total**	**Asa**	**Gad**	**Mdd**	**Sph**	**Pdis**	**Total**	**Alc**	**Cd**	**Odd**	**Drugs**
Fear 11	1																						
Frustration 11	.32*	1																					
Affiliation 11	.10*	-.16*	1																				
HighIntPl 11	-.22*	-.04	.13*	1																			
Shyness 11	.13*	.10*	-.31*	-.30*	1																		
Eff contr 11	-.23*	-.38*	.09*	-.09*	-.02	1																	
Fear 16	.44*	.23*	.06	-.12*	.08*	-.16*	1																
Frustration 16	.21*	.51*	-.10*	-.01	.03	-.28*	.39*	1															
Affiliation 16	.09*	.16*	.52*	.05	-.20*	.07*	.09*	-.16*	1														
HighIntPl 16	-.15*	-.10*	.14*	.59*	-.18*	.10*	-.20*	-.02	.13*	1													
Shyness 16	.09*	.10*	-.23*	-.21*	.58*	-.04	.19*	.13*	-.36*	-.29*	1												
Eff contr 16	.11*	-.30*	.09*	-.06	.02	.55*	-.19*	-.44*	.17*	.02	-.07*	1											
INT	.05	.03	.03	-.03	.01	-.04	.09*	.11*	.02	-.01	.02	-.05	1										
Adult sep anx	-.01	.01	.01	-.01	-.02	.03	.06*	.08*	.03	-.01	.01	-.04	.32*	1									
Gen anx dis	.03	-.01	-.01	.04	.04	-.02	.05	.02	.05	.02	.02	.01	.33*	.08*	1								
Maj depr dis	.05	-.01	.05	-.05	.02	-.02	.08*	.07*	.01	-.02	.02	-.04	.81*	.05	.08*	1							
Panic disorder	.04	.07*	.01	-.04	.00	.01	.06	.04	.02	-.02	.01	.03	.16*	-.01	-.01	.06*	1						
Social phobia	.01	.00	.03	-.02	.01	.01	.04	-.01	.06*	-.03	.02	.03	.27*	.10*	.20*	.11*	-.00	1					
EXT	-.01	.06*	.02	.09*	-.02	-.07*	.03	.12*	-.02	.03	-.02	.17*	.11*	.04	.01	.08*	-.01	.06*	1				
Alcohol dis	-.02	.03	.03	.07*	-.01	-.02	.02	.06*	-.00	.01	-.03	.06*	.03	-.02	-.02	.04	-.01	-.02	.65*	1			
Conduct dis	-.02	.03	-.00	.02	-.04	-.05	.00	.09*	.03	.06*	-.02	-.08*	-.02	-.01	-.01	-.02	-.00	-.01	.28*	.01	1		
Opp defiant	-.03	.06*	-.03	.01	-.01	-.02	.03	.08*	-.03	-.00	-.00	-.07*	.08*	.07*	-.01	.08*	-.01	.01	.38*	.03	.42*	1	
Drugs abuse	-.02	.01	-.01	.01	-.03	.02	.06*	.01	.07*	.00	-.03	-.03	.03	-.01	-.01	.04	-.00	-.01	.23*	.06*	.05	.01	1

Note. Values in bold reflect significant correlations, * = $p < .05$.

9

General summary and discussion

The aim of this PhD thesis was to investigate programming effects of adversity on temperament and HPA-axis functioning in adolescents. We started by elucidating one of the main temperament domains, neuroticism, to take a close look at stability and change of neuroticism in female twins. Then, we explored associations between our main outcome measures: temperament and HPA-axis functioning. Continuing with the key aims of this thesis, associations between adversity and changes in both temperament and HPA-axis functioning were addressed. Subsequently, we investigated inter-individual differences in sensitivity to adversity, and consequences of temperament change with regard to future stress exposure and psychiatric disorders. In this final chapter we summarise the findings and integrate them to provide a new perspective on stability and change in temperament and HPA-axis functioning.

Stability and change in temperament and HPA-axis functioning

Stability and normative change. It has long been noted that adaptive capacity (both in terms of temperament and HPA-axis functioning), as characterizing fundamental differences between people, is highly stable. Despite this stability assumption, there is now a growing body of evidence that some change is possible. With regard to temperament, numerous studies have pointed in this direction. First, longitudinal behavioural genetic studies have provided evidence for a 'change component' in addition to substantial stability (Kandler et al., 2010; Viken, Rose, Kaprio, & Koskenvuo, 1994). In Chapter 2 of this thesis we confirm and extend these findings. By disentangling the longitudinal structure of neuroticism in adult twins from the VATSPUD study, a change (or state) component was revealed that was about the same magnitude as the stability (or trait) component. However neuroticism tended to become increasingly stable across adulthood. Second, extensive literature has demonstrated mean level change in temperament (often operationalized as Big Five personality traits, (McCrae et al., 2002; Roberts, Wood, & Smith, 2005; Robins, Noftle, Trzesniewski, & Roberts, 2005). These studies, sometimes covering decades, have consistently revealed that a) change is possible until old age, but b) that there appears to be more mean-level change in adolescence and young adulthood than during any other period of the life course. Overall, changes have been found in the direction of maturation (e.g., decreases in emotional stability and increases in extraversion and conscientiousness with increasing age), suggesting that normative changes are characterized by maturation. Different theories have been proposed that may explain normative changes in temperament. One explanation for temperament maturation is the existence of a genetic characteristic that is shared species-wide (McCrae et al., 2002). Consequently, temperament change might simply be genetically driven. Both Kandler and colleagues (2010) and we (Chapter 2) have tested this Genetic Maturation Model, but found limited evidence. A second theory that has been proposed, and for which more evidence has been found in the literature (although it was not tested in the current thesis given our focus on adversity and non-normative development), is the Social Investment Principle. This

Social Investment Principle states that investing in social institutions, such as age-graded social roles like becoming a parent or getting a first job, is one of the driving mechanisms of temperament development (Roberts et al., 2005).

The findings of Chapter 4 support the notion of normative temperament change. Although this study was the first to look at normative changes in EATQ-R traits, our findings were comparable with changes in traits previously reported based on the Big Five. In line with the idea of development towards maturation, traits related to emotional instability (fear and frustration) decreased between age 11 and 16, whereas high intensity pleasure (related to extraversion) increased. Effortful control slightly decreased, which does not seem to reflect maturation, but have previously been suggested to be normative in the early adolescent years (Branje, van Lieshout, & Gerris, 2007). Similarly, decreases were found in affiliation, which might be normative given the age of our sample, and the use of parent-reports. In addition, rank-order change was explored, reflecting changes in the relative placement of individuals within a group. Studying differential stability can provide insights in individual variation in maturation Moderate test-retest coefficients were found for all traits, which is in line with findings of Roberts and DelVecchio (in a meta-analysis, Roberts & DelVecchio, 2000).

With regard to normative changes in HPA-axis functioning, far less is known. A single study has suggested mean level increases in basal cortisol during childhood, that leveled off during late adolescence (Trickett, Noll, Susman, Shenk, & Putnam, 2010). No studies at all seem to have investigated changes in stress-induced cortisol, although some cross-sectional studies have suggested stronger stress-induced cortisol activity in older than in younger adolescents (Gunnar, Wewerka, Frenn, Long, & Griggs, 2009). Using two waves of data of 141 adolescence (a subsample of TRAILS), we were the first exploring changes in basal cortisol, cortisol awakening responses and various measures of stress-induced cortisol (Chapter 5). Normative change was found to be larger in reaction to stress (i.e., substantial increases were demonstrated) compared to the other cortisol measures, confirming both the longitudinal findings on basal and the cross-sectional findings on stress-induced cortisol. It should be noted, however, that although increases in stress-induced cortisol seem to be normative during adolescence, it is unclear whether these changes are an indication of maturation, that is, whether a stronger reaction to stress has any age-graded adaptive value. Future research should study this in more detail, for example in the light of coping with environmental challenges.

Subsequently, we examined differential stability in the various cortisol measures. Studying differential stability of HPA-axis functioning is novel to the literature and can provide new insights in individual variation in maturation of the HPA-axis. Differential stability was substantial, although lower than has usually been found for temperament measures. This may indicate that HPA-axis functioning is less stable, or and maybe more plausible, measures of HPA-axis functioning may include more noise than most temperament questionnaires. That is, measures of HPA-axis functioning may be more strongly influenced by confounders such as food intake, use of medication and sleep, than temperament (probably resulting in a general underestimation of the effects reported in this thesis).

Non-normative change and adversity. Despite the numerous studies that extensively described normative temperament development, changes in traits have rarely been studied in depth. That is, limited longitudinal research has been performed on variables related to, or mechanisms underlying, these changes. With regard to HPA-axis functioning longitudinal studies on environmental influences seem to be lacking completely. One plausible explanation for this apparent gap in the literature might be the stability assumption of adaptive capacity. The assumption that temperament and HPA-axis functioning are highly stable characteristics that only show modest changes in the direction of maturation, may implicitly entail that non-normative change is very small, if not redundant.

A few studies have investigated non-normative changes in temperament, in particular in traits related to emotional stability. Findings suggested that non-normative change may have its origins in major, traumatic life events such as loss of a loved one (Mroczek & Spiro, 2003). Thus, whereas normative temperament change may be a result of normative life-events, such as getting a responsible job, non-normative change may be a result of non-normative, idiosyncratic events, such as death of significant others. However, the findings by Mroczek and Spiro have only incidentally been replicated (Löckenhoff, Terracciano, Patriciu, Eaton, & Costa, 2009; Vaidya, Gray, Haig, & Watson, 2002) and were based on adults, whereas also other age groups may be interesting to study. Given that adolescence is a period characterised by major biological, psychological and social changes, intense interactions with the environment and increased brain plasticity (Lupien, McEwen, Gunnar, & Heim, 2009), it might be that environmental influences such as adverse events have more severe and maybe more enduring consequences than during adulthood.

In Chapter 4 and Chapter 5 we examined the association between adversity (i.e., adverse events) and changes in respectively temperament and HPA-axis functioning during adolescence. Our findings revealed that whereas normative temperament change occurs often in the direction of maturation, adolescents exposed to adverse events show less maturation of their temperament or even the reverse of maturation. Similarly, whereas adolescents not exposed to adverse events showed increases in stress-induced HPA-axis activity, adolescents exposed to social defeat (e.g., being a victim of violence), showed similar reactivity at both waves. These findings are probably the most important ones reported in this thesis. The finding that adversity is related to non-normative changes in both temperament and HPA-axis functioning provides evidence that contradicts the assumptions of stability and universal development towards maturation.

Theoretical frameworks for adversity and changes in adaptive capacity

The Scar Model. Our findings that adolescents exposed to adversity do not show the normative changes in temperament and HPA-axis functioning shown by other adolescents. This can be interpreted in the light of the Scar Model, which argues that, analogous to the scar tissue that will never become like normal skin again, people who have experienced an adverse event (i.e., death of a friend), will never be the same as before (e.g., Zeiss & Lewinsohn, 1988). Originally,

the Scar Model has been developed to explain the association between adverse events and depression, but it may also provide a theoretical base for the association between adverse events and changes in temperament and HPA-axis functioning.

The results in our study seem to confirm the idea that adversity can alter relatively fundamental characteristics (apparently also characteristics more fundamental than originally proposed by Zeiss and Lewinsohn in their research on stress and depression). However, it might be that the impact of adversity on temperament is not as irreversible as the Scar Model suggests. Instead, adverse events may result in more temporary changes in temperament and HPA-axis functioning. Although the data used in Chapter 4 and 5 covered multiple years, which is more than any other study so far on HPA-axis functioning, the time span is not large enough to be conclusive with regard to the irreversibility of the associations found. Some support for stress affecting changes in temperament for a longer period of time than covered by our study, however, can be found in research with adults. Having experienced an extremely adverse event has recently been found to be related to increases in traits related to emotional instability, over an eight-year period (Löckenhoff et al., 2009). Future studies including multiple waves of adverse events, HPA-axis functioning and temperament, and covering a larger time span than ours would be needed to investigate the Scar Model as a theoretical framework for the association between adverse events and temperament or HPA-axis functioning in more detail.

In addition, it should be noted that whereas exposure to adverse events was related to changes in all temperament traits under study (although associations were stronger for traits related to emotional stability than for the other temperament traits), this was not the case for the associations between adversity and HPA-axis functioning. Adversity was related to stress-induced HPA-axis reactivity, but not to the other cortisol measures under study (see also the paragraph on generalizability and specificity). Furthermore, in Chapter 4 we used a cumulative stress index including both mild and more severe events. Our findings showed a clear linear trend between the number of adverse events an adolescent was exposed to and the amount of temperament change shown, suggesting that even milder events can affect temperament change. In contrast, in Chapter 5, we distinguished between two types of relatively severe stressors: loss/illness of significant others and social defeat (e.g., being a victim of violence; 180 adolescents were selected to participate in the Social Stress Task at age 19 based on their exposure to adverse events because random selection from the TRAILS sample would lead to a sample in which only a few individuals would have been exposed to adversity, and subsequently, to power problems). The findings revealed that only social defeat (and not loss/illness) was related to HPA-axis functioning. The fact that only severe, integrity threatening events were related to changes in HPA-axis functioning may reflect a larger resistance of HPA-axis functioning against environmental influences. Another explanation might be found in the lower test-retest stability of HPA-axis functioning. If measures of HPA-axis functioning are more sensitive to confounders than measures of temperament, and subsequently include more noise, it is plausible that the reported effects of adversity on changes in HPA-axis

functioning underestimate the real associations compared to the effects of adversity on changes in temperament.

The Corresponsive Principle. The Scar Model departs from the stress-effect principle, which assumes that adversity can affect adaptive capacity (either in terms of temperament or in terms of HPA-axis functioning), but disregards the possibility that adaptive capacity can also affect subsequent stress exposure. A model that may account for the possible bidirectional associations between adversity and adaptive capacity is the Corresponsive Principle. The Corresponsive Principle hypothesizes that change in adaptive capacity, for example temperament development, is the result of mutually reinforcing person–environment transactions including two processes: 1) *social selection* (people select environments that correlate with their temperament) and 2) *social influence (*these environments produce experiences that influence temperament; Caspi, Roberts, & Shiner, 2005). Thus, this model suggests that adversity does not only predict subsequent temperament or HPA-axis functioning, but that temperament or HPA-axis functioning can also predict subsequent exposure to adversity.

The findings reported in Chapter 6 partly confirm this notion. By modeling three waves of temperament and adversity data, we could disentangle the direction of the effects between adversity and temperament. Our findings showed that the longitudinal associations between adversity and different temperament traits varied across traits. Adolescents who were exposed to more adverse events showed higher subsequent levels of fear and frustration. However, no evidence was found for either fear or frustration predicting subsequent adverse events, providing support for the, often assumed, stress-effect model. The opposite pattern was found for affiliation and shyness, the two traits related to the domain of extraversion. Adolescents who were high on affiliation or low on shyness were more likely to be exposed to subsequent adverse events. Only for effortful control, related to the domain of conscientiousness, the longitudinal association with adverse events was bidirectional, low effortful control predisposed adolescents to experience more adverse events and exposure to adverse events predicted lower levels of subsequent effortful control. Taken together, although we did not find much support for bidirectional associations (that is, both selection and causation) between stress and most of our temperament traits, our findings clearly support the importance of taking into account the direction of the effects. While the stress-effect model may follow naturally from the established associations between stress and psychopathology, and between traits and psychopathology, this model could only account for longitudinal associations between stress and fear and frustration. A trait-effect model seemed to reflect the association between adverse events and affiliation and shyness, much more adequately because adolescents high on traits related to extraversion may interact more with their environment and therefore experiences more events (both negative and positive). A bidirectional, or corresponsive model was only found for the association between stress and effortful control. Adolescents high on effortful control, related to conscientiousness, may have more control over their life and are less likely to get into trouble. Subsequently, low levels of

adversity may be related to more feelings of control over one's life and subsequently to higher scores on measures of effortful control.

Additional support for the corresponsive principle has been reported in Chapter 2 and Chapter 7 of this thesis. Our finding that stability of neuroticism increased across waves and with age (Chapter 2), as well as the fact that the environmental component was larger in older than in younger twins, is in line with both social selection and social influence. In Chapter 7, we investigated the longitudinal, reciprocal associations between stressful events and psychological difficulties from early childhood to mid-adolescence using the British ALSPAC data. As it turned out, there was consistent evidence of a bidirectional pattern: children exposed to stressful events showed significantly increased psychological difficulties a few years later and psychological difficulties predicted subsequent stressful events.

In the current thesis we did not investigate the association between adverse events and HPA-axis functioning in the light of the corresponsive principle. Although our two-wave design is novel to the literature, three waves would be needed to elucidate the longitudinal bidirectional associations in a statistically robust way. Probably, the findings from Chapter 2 and 6 do not simply generalize to the association between adverse events and HPA-axis functioning. Although we perceive temperament and HPA-axis functioning both as levels at which adaptive capacity can be studied, as reported in Chapter 3, the association between temperament and HPA-axis functioning is limited to basal cortisol and some facets of temperament. Subsequently, future research may explore if and to what extent the corresponsive principle holds for the association between adverse events and HPA-axis functioning.

Generalizability versus specificity. The theoretical frameworks described above may not be mutually exclusive. Instead, the associations between adverse events and changes in temperament and HPA-axis functioning are probably a mix of processes. However, not all associations are equally captured by the two models. For example, the Scar Model (or stress-effect/causation) may best reflect the association between adverse events and emotional instability, whereas the association between adverse events and effortful control is better reflected by the Corresponsive Principle (or bidirectional model). Similarly, adverse events were related to changes in HPA-axis reactivity induced by a social stress task, but not to changes in basal cortisol, cortisol awakening response, anticipation to and recovery after the stress task and total cortisol output during the task. Additionally, with regard to the associations between temperament and HPA-axis functioning, basal cortisol, but none of the other cortisol measures, was related to most, but not all, temperament traits.

These findings raise the issue of generalizability. It may seem that in each chapter of this thesis the associations reported exist only for some of the variables under study. However, when combining the different results and integrating them in the existing literature, some trends become clear. First, the stronger association between basal cortisol and temperament, than between temperament and the other cortisol measures, may be a result of the trait-like nature of both basal cortisol and temperament (Bartels, Van den Berg, Sluyter, Boomsma, &

de Geus, 2003; Federenko, Nagamine, Hellhammer, Wadhwa, & Wüst, 2004; Hellhammer et al., 2007; Wüst, Federenko, Hellhammer, & Kirschbaum, 2000). Stress-induced cortisol reactivity, in contrast, has been suggested to be a more state-like characteristic, which might explain 1) the lack of association with temperament and 2) why it is affected by exposure to social defeat (in contrast to basal cortisol, and despite the relatively low test-retest correlation that may mask part of the association with adversity). With regard to the association between adverse events and temperament, the temperament traits (fear and frustration) that were most strongly affected by adversity were those that have been reported to be most consistently associated with psychopathology. Given the strong associations respectively between emotional instability and psychopathology, and between adverse life events and psychopathology, the strong effects of adverse events on traits related to emotional instability seem plausible.

Taken together, even though our findings seem to complement each other quite well, they clearly provide support for the notion of specificity. Taking into account specificity seems to be relatively new to the literature on adversity and adaptive capacity. Whereas research often tends to study temperament at the level of broad traits like the Big Five, our findings show the additive value of studying traits at the facet level (e.g., both fear and frustration as facets of emotional instability). Similarly, whereas research on HPA-axis functioning often focuses on a single measure (e.g., basal cortisol or reactivity to a stress task), our findings demonstrate that the associations between adversity and HPA-axis functioning vary dependent on the cortisol measures under study. Thus, although the availability of multiple temperament facets and various cortisol measures may not directly lead to a clear-cut picture, it may be one of the main strengths of this thesis. It would be highly interesting for future research to include multiple measures as well, to see whether and to what extent our findings replicate to other samples, age groups and instruments. For example, other physiological characteristics involved in stress-responses (e.g., cardiovascular stress-reactivity) would be interesting to include in future (TRAILS) studies. More important, if inclusion of an elaborate temperament questionnaire, or multiple measures of HPA-axis functioning, is not feasible, based on our findings it might be recommendable to carefully consider which measures are the most valuable to include based on the study hypotheses.

Inter-individual differences in sensitivity to adversity

Over the last decade an increasing emphasis has emerged on the possibility that certain factors can explain differences in sensitivity to adverse events. More specific, individual characteristics make certain individuals consistently more sensitive to environmental influences than other individuals, possibly for better and certainly for worse. As described in the introduction of this thesis, the notion of differential sensitivity has been studied in the light of the Diathesis Stress Model (Monroe & Simons, 1991), assuming that risk exposure may accumulate and amplify the impact of (subsequent) stress on sensitive individuals and, more recently, in the light of the Differential Susceptibility Model (Belsky & Pluess, 2009). This latter model proposes that sensitive individuals may not only suffer more from adversity, but additionally, benefit

more from a supportive environment than less sensitive individuals. Similarly, the Biological Sensitivity to Context Model (Boyce & Ellis, 2005; Ellis & Boyce, 2008) proposes postnatal programming of the physiological stress response system, depending on the environment (adverse versus supportive) a young child first experiences. Given the focus on adversity of this thesis, we did not address the notion of sensitivity to a supportive environment. However, several candidates were explored that may explain differences in sensitivity to adverse events. Little evidence was found for either sex (Chapter 4) or age (Chapter 6) explaining differences in sensitivity, though these moderators have previously been studied in the context of adverse events and psychopathology. Additionally, in Chapter 6 we explored various candidates new to the literature. First, we tested the whether the association between stress and subsequent temperament was moderated by a cumulative genetic plasticity index as proposed by Belsky and Beaver (2011). The use of such an index, reflecting the number of plasticity alleles that have been suggested as a source of sensitivity, is novel and may solve the power problems that have been encountered by studies focusing on only one or two plasticity alleles. Nonetheless, despite this rationale to use a plasticity index, we did not find any effect on sensitivity to adverse events. Although some studies have suggested that the same genes act on temperament as on psychopathology, it might be that other genes, acting on different physiological systems, play a more important role in the association between adverse events and temperament than the genes we included. In addition, and as previously described in a TRAILS study by Stavrakakis and colleagues (Stavrakakis et al., 2012), the exact functioning of certain polymorphisms is not entirely clear, which may make combining different plasticity genes problematic.

Another candidate of sensitivity we examined was prenatal adversity. Although adolescents high on prenatal adversity seemed somewhat more sensitive to the influence of adversity, the effects were not consistent. The lack of a consistent moderator effect of prenatal adversity confirms and extends the finding of Chapter 7 that prenatal anxiety does not moderate the longitudinal association between childhood stress and psychological difficulties.

Although we did not find an effect of prenatal anxiety, both sex and age effects moderated the association between adverse events and psychological difficulties, which is in line with previous studies on stress and psychopathology (Grant et al., 2006; Lupien et al., 2009; Oldehinkel & Bouma, 2011). The finding that sex and age did not moderate the association between adversity and temperament, but did moderate the association between adversity and psychological difficulties warrants interpretation and further research. It might be that the trait-like nature of temperament makes less vulnerable (although not completely resistant) to inter-individual differences in sensitivity to adversity than psychological difficulties. Psychological difficulties, in contrast, may be approached as surface traits. That is, they are more state-like and closer to psychopathology than temperament, which may make them more open to inter-individual differences in sensitivity to adversity. Alternatively, the nature of differential sensitivity might be more complex with respect to the association

between adversity and temperament than regarding adversity and psychopathology, and subsequently, differences in sensitivity to the effect of adversity on temperament may only exist in terms of geneXenvironment or personXenvironment interactions. Of course, this hypothesis needs further examination before we can be conclusive on this issue.

However, some evidence for the complex nature of differences in sensitivity to the association between adversity and temperament was found in the preliminary analyses of Chapter 6. As it turned out, bivariate correlations between stressful events and both fear and frustration were much stronger in adolescents high on both plasticity genes ánd prenatal adversity, than in adolescents low on both or high on either plasticity genes or prenatal adversity. It might be that genes were turned on by early adversity, and thus, if an individual was high on plasticity genes, this might have resulted in an increased sensitivity and a stronger reaction to stressful events later in life. This hypothesis would be in line with the increasingly popular notion of epigenetics (Bernstein, Meissner, & Lander, 2007; Charney, 2012). However, due to power issues (n = 34 for the adolescents high on plasticity genes and prenatal adversity), the difference between adolescents high on both plasticity genes and prenatal adversity and the other adolescents was not significant in the more conservative cross-lagged multi-group analyses, and caution with interpretation is needed. Future research within TRAILS or in another large cohort, extending the current study design with a traditional epigenetics approach (that is, an approach in which activity of alleles is physiologically measured), could test whether our findings can indeed be explained by activation of (plasticity) genes in the prenatal period.

Consequences of changes in adaptive capacity

When studying associations between adversity and changes in temperament and HPA-axis functioning, the question rises whether change matters. Is temperament change, for example, predictive of anything relevant, or are we talking about normative variations in adolescent development? As described earlier in this chapter, in Chapter 6 and Chapter 7 we showed that both temperament and psychological difficulties can be predictive of future stress exposure. Chapter 8 extended this study by addressing the question whether changes in temperament are predictive of mental disorders a few years later. Although extensive literature has provided evidence for a cross-sectional association between temperament and psychopathology (Kotov, Gamez, Schmidt, & Watson, 2010), and a few studies has examined the longitudinal relationships between the two (e.g., Klimstra, Akse, Hale III, Raaijmakers, & Meeus, 2010), we seem to be the first predicting psychiatric diagnoses prospectively from temperament change. The results revealed that temperament change has an additional effect on future disorders, above and beyond basal temperament. This finding elaborates traditional tests of the Vulnerability Model (which states that personality can place individuals at risk for the development of mental disorders), predicting mental disorders from a single temperament measure (Tackett, 2006). Moreover, the results provide insight in the long term consequences of temperament change on adolescent mental health. However, the study is only one way

to address the question whether change matters. Other outcome measures, such as school or work functioning might be interesting to look at as well. In addition, in the current thesis we did not examine long-term consequences of changes in HPA-axis functioning, whereas this would provide novel insights in the meaning and implications of adversity-induced blunting of the physiological stress-system. Further research would be required to increase our knowledge in this topic.

A new perspective on adversity and adaptive capacity: scientific implication and future directions

Throughout this thesis we have provided evidence that exposure to adverse events is related to non-normative changes in temperament and HPA-axis functioning. This could well be the most important finding, calling into question the basic assumptions of stability and maturation of adaptive capacity. The findings indicate that temperament and HPA-axis functioning should be studied in the context of (adverse) life-experiences and not just as stable predictors or in terms of normative changes towards maturation. Moreover, the importance of environmental influences was not only revealed in our TRAILS studies on adversity and adaptive capacity (Chapter 4-6), but also in Chapter 2 and Chapter 7, in which we used data from two other large cohort studies: VATSPUD and ALSPAC.

Still, one might be concerned that our findings may not generalize to other measures or other samples. In particular the selection of adverse events may give rise to questions. To measure adverse events we used a (standardized) count score of events that were measured within TRAILS (and ALSPAC) and that have previously been suggested as relevant adverse events during adolescence. However, some adolescents may be exposed to more, more severe, or other events than the ones we included. Indeed, we found differential effects of social defeat and loss on changes in HPA-axis functioning (Chapter 5), suggesting specificity in the associations as well as the importance of considering the nature of the adverse events under study when formulating hypotheses. Nonetheless, given the linear trends reported in Chapter 4, suggesting that both mild and more severe events can contribute to changes in temperament, it seems that our findings only reveal the top of the iceberg. Including more or more severe events would probably increase the strength of the association between adverse events and temperament change, because they might, more than milder events, have the potential to cause changes in adaptive capacity.

Even if effects of adversity on changes in temperament and HPA-axis functioning are very small, we think that they deserve interest. First, whereas exposure to only one, or a relatively minor event will probably result in a very small change, Chapter 2, 4 and 7 suggest the presence of accumulation of effects and a vicious cycle. Adverse events can alter an individual's temperament, which in turn makes that the individual selects an environment that may be characterized by high stress. This way, adversity can both result in non-normative temperament change (less or even the reverse of maturation) and maintain the individuals' (maladaptive) temperament. Moreover, the more events an individual is exposed to, the larger the deviations from normative temperament change. Thus, initially small effects

may accumulate in the long run and therefore interfere with normative temperament maturation. Second, temperament has been assumed to be one of the most stable concepts in psychology. Given the strong and well established effects of temperament on various domains of functioning (mental health, career success etc.), it seems plausible that any non-normative temperament change can have substantial consequences. The same might hold for changes in HPA-functioning. Although in Chapters 6 and 8 we made a start with exploring the consequences of temperament change, there is much to be revealed. An interesting goal for future research would be to further investigate and disentangle the consequences of (adversity driven) changes in temperament and HPA-axis functioning.

The longitudinal approach in most chapters is clearly one of the major strengths of this thesis. In particular with regard to HPA-axis functioning, only a few longitudinal studies (on basal cortisol) have been performed so far. The findings reported in this thesis, especially those based on the repeated measures of the social stress task, may therefore substantially add to our knowledge. However, despite the multiple waves and years covered in the current design, some questions remain that might be answered in future studies. Most important, although our findings provide support for some scar effects and maybe even a negative spiral, little can be said about the long-term impact of adversity on adaptive capacity. Three hypotheses might be worth testing with regard to the long-term effects of adversity. First, and most likely based on our findings, the impact of adversity on temperament and HPA-axis functioning prolongs, due to both causation and selection, resulting in a vicious cycle of stress exposure and non-normative changes in temperament and HPA-axis functioning. Second, the impact of adversity on temperament and HPA-axis functioning lasts for a few years, but fades away due to other (positive) environmental influences or new social roles that have stronger effects on temperament than the adverse events that occurred during adolescence. Finally, although beyond the scope of this thesis, it might be interesting to study possible positive consequences of (adversity-driven) changes in temperament and HPA-axis functioning. Could it be that there are any positive effects? For example in terms of coping with daily hassles? In the end, the notion of temperament and HPA-axis functioning as levels of adaptive capacity implies they are not only involved in risk, but also in resiliency and (mental) well-being.

Conclusion

The title of this thesis proclaimed that our research would deal with programming effects of adversity. Indeed, the main findings point out that exposure to adversity is related to non-normative changes in temperament and HPA-axis functioning during adolescence. Limited evidence was found for differential sensitivity to the effects of adversity. Findings described consequences of temperament change, both in terms of future stress exposure and in terms of future mental disorders. Taken together, these findings contradict the traditional stability assumption, extend descriptive studies on normative changes towards maturation, and most important, emphasize the importance of studying changes in temperament and HPA-axis functioning in the context of (adverse) life events.

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Nederlandse samenvatting

Stressvolle gebeurtenissen zijn een bekende risicofactor voor de ontwikkeling van psychische aandoeningen. Uit onderzoek is gebleken dat psychische aandoeningen vanaf het begin van de adolescentie vaker voorkomen. Deze stijging, in combinatie met de biologische, psychische en sociale veranderingen die kenmerkend zijn voor de adolescentie, leidt tot de vraag of de adolescentie kan worden gezien als een gevoelige periode wat betreft de invloed van stress. De afgelopen jaren hebben onderzoekers een toenemende interesse ontwikkeld in de mechanismes die de relatie tussen stress en psychische gezondheid kunnen verklaren. Een belangrijk onderliggend mechanisme zou door stress veroorzaakte verandering in adaptieve capaciteit kunnen zijn. Adaptieve capaciteit weerspiegelt constitutionele verschillen tussen mensen en kan iets zeggen over iemands vermogen zich aan te passen aan een (veranderende) omgeving. In dit proefschrift staat centraal of en hoe blootstelling aan stressvolle gebeurtenissen kan leiden tot veranderingen in adaptieve capaciteit.

Achtergrond

Adaptieve capaciteit kan op verschillende niveaus worden bestudeerd. Misschien wel het meest bestudeerde niveau is temperament. Lange tijd werd temperament gezien als een, voornamelijk biologische, basis voor iemands latere persoonlijkheid. Tegenwoordig zijn onderzoekers het er steeds meer over eens dat temperament en persoonlijkheid equivalenten van elkaar zijn: beiden weerspiegelen relatief stabiele verschillen tussen personen. In dit proefschrift worden de termen door elkaar gebruikt. Naast temperament kan adaptieve capaciteit ook bestudeerd worden op een fysiologisch niveau, zoals met betrekking tot cardiovasculair functioneren of functioneren van de hypothalamus-pituitary-adrenal as (HPA-as). Dit proefschrift richt zich op functioneren van de HPA-as. De HPA-as is een belangrijk mechanisme in lichamelijke stressreacties. Door blootstelling aan stress wordt de HPA-as geactiveerd. Hierdoor komt het (stress)hormoon cortisol vrij. De stijging in cortisol vanaf het moment dat iemand ontspannen is tot het moment van stress, zegt iets over hoe sterk zijn of haar lichamelijk reactie op de gebeurtenis is. Voor dit proefschrift is cortisol gemeten op verschillende momenten waaronder in rust en tijdens het uitvoeren van een stressvolle taak in het laboratorium. Zowel temperament als functioneren van de HPA-as zijn in het verleden voorspellend gebleken voor de ontwikkeling van psychische aandoeningen, alhoewel de bevindingen consistenter lijken te zijn voor temperament dan voor de HPA-as.

Het begrip adaptieve capaciteit impliceert een zekere mate van stabiliteit. De afgelopen decennia is echter gebleken dat temperament toch enigszins kan veranderen. Over stabiliteit en verandering in HPA-as functioneren is minder bekend, omdat er maar weinig onderzoek is gedaan waarbij HPA-as functioneren meerdere keren is gemeten. Nog minder is er bekend over de rol van omgevingsinvloeden, zoals stressvolle gebeurtenissen, bij veranderingen in adaptieve capaciteit. Uit onderzoek is gebleken dat stress gerelateerd is aan veranderingen in temperament bij volwassenen. Het is onduidelijk of dit ook voor adolescenten geldt. Bovendien is het onduidelijk op welke temperamentstrekken stress tijdens de adolescentie

een effect kan hebben en of het meemaken van meerdere gebeurtenissen ook tot meer verandering in temperament leidt. Wat betreft de HPA-as hebben verschillende studies een relatie aangetoond tussen stress en HPA-as functioneren (gemeten op hetzelfde moment). Het is echter onbekend of stress ook daadwerkelijk kan leiden tot *veranderingen* in HPA-as functioneren, en zo ja, of de invloed hetzelfde is voor verschillende soorten stressvolle gebeurtenissen en voor verschillende maten van HPA-as functioneren.

Gerelateerd aan de vraag of stress adaptieve capaciteit kan beïnvloeden is de vraag of de invloed van stress voor iedereen hetzelfde is. De afgelopen jaren is er bij onderzoekers een sterke interesse ontstaan in de vraag of, en waarom, sommige mensen gevoeliger zijn voor de invloed van stress dan andere. Ondanks een overdaad aan onderzoeken is er echter nog veel onduidelijk over wat deze verschillen veroorzaakt. Bovendien hebben de meeste studies tot nu toe gekeken naar verschillen in gevoeligheid voor de invloed van stress op de ontwikkeling van psychische aandoeningen en niet naar verschillen in gevoeligheid voor de invloed van stress op veranderingen in adaptieve capaciteit.

Bij het onderzoeken naar de relatie tussen stress en veranderingen in adaptieve capaciteit komt de volgende vraag naar voren: Zijn (door stress veroorzaakte) veranderingen in adaptieve capaciteit schadelijk? Maakt het uit als, bijvoorbeeld, de temperamentontwikkeling niet bij alle adolescenten hetzelfde is? Uit tientallen jaren van onderzoek is duidelijk gebleken dat temperament gerelateerd is aan psychische aandoeningen. Het is echter nog onbekend of veranderingen in temperament een adolescent ook gevoeliger maken voor de ontwikkeling van een (nieuwe) aandoening enkele jaren later.

Het doel van dit proefschrift is om bij te dragen aan het ontrafelen van de complexe relatie tussen stressvolle gebeurtenissen en veranderingen in adaptieve capaciteit bij adolescenten. Hierbij staan twee niveaus van adaptieve capaciteit centraal: temperament en HPA-as functioneren. Er is onderzocht hoe verschillende soorten stress verschillende effecten kunnen hebben en hoe stress sommige temperamentstrekken en sommige maten van HPA-as functioneren sterker beïnvloedt dan andere. Er is onderzocht of stress alleen temperament beïnvloedt, of dat adolescenten met een bepaald temperament ook meer kans hebben stressvolle gebeurtenissen mee te maken. Er is ook gekeken of deze relaties voor iedereen hetzelfde zijn of dat sommige adolescenten gevoeliger zijn dan andere. Tot slot is er gekeken naar de lange termijn effecten van veranderingen in temperament op de ontwikkeling van psychische aandoeningen.

Gebruikte data

Voor het onderzoek in dit proefschrift is gebruik gemaakt van data van TRAILS (TRacking Adolescent's Individual Lives Survey). TRAILS is een cohortonderzoek onder 2230 adolescenten uit Noord Nederland. Deze adolescenten worden om de 2 á 3 jaar geïnterviewd, vanaf hun 11de totdat ze tenminste 25 jaar oud zijn. Behalve de adolescenten zelf zijn ook hun ouders, broers/zussen, leraren en klasgenoten ondervraagd over de (geestelijke) gezondheid, school, vrienden, enzovoorts. Stressvolle gebeurtenissen zijn uitgebreid uitgevraagd met behulp

van gestandaardiseerde interviews. Temperament is 3 keer gemeten door middel van vragenlijsten, toen de adolescenten 11, 16 en 19 jaar oud waren. Functioneren van de HPA-as is gemeten in speeksel toen de adolescenten 16 jaar oud waren, en opnieuw toen ze 19 jaar waren. Dit gebeurde zowel thuis (vlak na het wakker worden) als tijdens een stressvolle taak in het laboratorium waarbij de deelnemers de opdracht kregen om een presentatie te geven en moeilijke rekensommen te maken. Daarnaast is gebruik gemaakt van data van de Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPUD; hoofdstuk 2) en van de Avon Longitudinal Study of Parents and Children (ALSPAC; hoofdstuk 8).

Stabiliteit en verandering in temperament en HPA-as functioneren

Zowel studies met tweelingen als longitudinale studies hebben aangetoond dat adaptieve capaciteit niet zo stabiel is als lang werd gedacht. Het onderzoek in hoofdstuk 2 bevestigt deze bevindingen. In dit hoofdstuk, waarvoor gebruik is gemaakt van de Amerikaanse tweelingen dataset VATSPUD, wordt bewijs gevonden voor zowel stabiliteit als verandering in neuroticisme (emotionele instabiliteit). Daarnaast laat het onderzoek zien, dat stabiliteit en verandering allebei door zowel genen als door de omgeving beïnvloed worden en dat stabiliteit groter is bij oudere tweelingen dan bij jong volwassenen. In de studie beschreven in hoofdstuk 4 is gekeken naar veranderingen in temperament in TRAILS. Net als in eerdere studies vinden we bewijs voor normatieve verandering. Ofwel, gedurende de adolescentie ontwikkelen jongeren over het algemeen een meer 'volwassen' temperament (o.a., en in overeenstemming met het onderzoek in hoofdstuk 2, dalingen in emotionele instabiliteit). Gezien de bevinding beschreven in hoofdstuk 2 is het waarschijnlijk dat deze normatieve veranderingen het gevolg zijn van zowel genetische als van omgevingsfactoren. Wat betreft stabiliteit en verandering in HPA-as functioneren: de bevindingen beschreven in hoofdstuk 5 suggereren dat verandering tijdens de adolescentie mogelijk is. Hierbij moet echter de kanttekening worden gemaakt, dat er nog maar zo weinig longitudinale studies zijn naar HPA-as functioneren dat het lastig is om te bepalen of de veranderingen die wij vonden belangrijk zijn voor de ontwikkeling van de adolescent (bijvoorbeeld dat een sterkere reactie op stress een indicatie is van maturatie).

Normatieve veranderingen en de rol van stressvolle gebeurtenissen

Hoewel normatieve veranderingen in temperament in het verleden al uitgebreid bestudeerd zijn was er tot nu toe weinig bekend over de oorzaken van verandering. Met betrekking tot veranderingen in HPA-as functioneren is er zelfs nog minder bekend. Het ontbreken van onderzoek op dit gebied is mogelijk het gevolg van de veronderstelling dat adaptieve capaciteit niet of nauwelijks verandert. Als temperament en HPA-as functioneren stabiel zijn, of alleen enkele normatieve veranderingen richting volwassenheid laten zien, is het aannemelijk dat non-normatieve veranderingen (bijvoorbeeld als gevolg van blootstelling aan stress) heel klein of zelfs verwaarloosbaar zijn.

De paar studies naar stressvolle gebeurtenissen en temperament (meestal emotionele instabiliteit) die gedaan zijn bij volwassenen hebben echter laten zien dat stress wel degelijk temperament kan beïnvloeden. Onze resultaten bevestigen deze bevindingen. Uit de studies die beschreven zijn in hoofdstuk 4 en 5 blijkt, dat stress ook de ontwikkeling van temperament en HPA-as functioneren tijdens de adolescentie kan beïnvloeden. De bevindingen laten zien dat, terwijl normatieve ontwikkeling meestal in de richting van een meer volwassen temperament gaat (maturatie), adolescenten die stressvolle gebeurtenissen hebben meegemaakt minder maturatie van hun temperament laten zien. Vergelijkbaar hiermee vinden we dat adolescenten die weinig stressvolle gebeurtenissen meemaken, een grotere lichamelijke reactie op een stresstaak in het laboratorium laten zien op 19 dan op 16-jarige leeftijd (dat is, een sterkere stijging in cortisol in reactie op de stresstaak), terwijl adolescenten die tussen hun 16de en 19de jaar blootgesteld waren aan bepaalde stressvolle gebeurtenissen, een zelfde reactie lieten zien op 19 als op 16-jarige leeftijd. Deze bevindingen zijn misschien wel de belangrijkste van dit proefschrift. De conclusie dat stressvolle gebeurtenissen gerelateerd zijn aan non-normatieve veranderingen in zowel temperament als HPA-as functioneren is in tegenspraak met de assumpties van stabiliteit en normatieve veranderingen richting volwassenheid.

Bovenstaande bevindingen lijken te kloppen met het 'Scar model', een theoretisch model dat oorspronkelijk werd gebruikt voor de relatie tussen stressvolle gebeurtenissen en depressie. Dit model stelt dat, net zoals littekenweefsel nooit meer helemaal hetzelfde wordt als gezonde huid, mensen die een stressvolle gebeurtenis hebben meegemaakt nooit meer helemaal dezelfde persoon zullen worden als voor de gebeurtenis. Hoewel deze metafoor juist lijkt wat betreft de invloed die (sommige soorten) stress kan hebben op temperament en HPA-as functioneren, doet hij te kort aan de complexiteit van de relatie.

Zoals beschreven in hoofdstuk 6 van dit proefschrift heeft stress niet alleen effect op adaptieve capaciteit, maar kunnen bepaalde temperamentstrekken er ook toe leiden dat een adolescent een grotere kans heeft op het meemaken van stressvolle gebeurtenissen in de toekomst. Zo laat het onderzoek in hoofdstuk 6 zien dat 1) jongeren die blootgesteld zijn aan stress, enkele jaren daarna minder goed hun aandacht kunnen reguleren en 2) dat jongeren die moeite hebben met het reguleren van aandacht ook een grotere kans hebben op het meemaken van stressvolle gebeurtenissen enkele jaren later. Dus, de relatie tussen stress en adaptieve capaciteit wederkerig zijn (het 'Corresponsive principle'). In de studie beschreven in hoofdstuk 7 vinden we vergelijkbare relaties; stressvolle gebeurtenissen voorspellen psychologische problemen enkele jaren later, maar kinderen en adolescenten die veel psychologische problemen hebben, hebben ook een grotere kans om stressvolle gebeurtenissen mee te maken. Ondersteuning voor dit principe is ook beschreven in hoofdstuk 2, waarin gevonden werd dat stabiliteit van neuroticisme groter is in oudere dan in jongere tweelingen, als ook dat de invloed van de omgeving groter is bij oudere dan bij jongere tweelingen.

In dit proefschrift hebben we niet kunnen onderzoeken of ook de relatie tussen stressvolle gebeurtenissen en HPA-as functioneren wederkerig is. Om dat te kunnen doen zouden minstens drie meetmomenten nodig zijn. Hoewel we HPA-as functioneren en temperament allebei als een niveau van adaptieve capaciteit zien, is het maar de vraag of de relaties met stress vergelijkbaar zijn. Zoals beschreven in hoofdstuk 3, is de relatie tussen HPA-as functioneren en temperament zwak, en beperkt tot functioneren tijdens rust (niet tijdens de stresstaak) en tot slechts enkele temperamenttrekken.

Individuele verschillen in gevoeligheid voor stress

In het afgelopen decennium zijn er tientallen, zo niet honderden studies gedaan naar of en waarom mensen verschillen in gevoeligheid voor stress. De bevindingen in dit proefschrift (hoofdstuk 4, 6 en 7) bevestigen wat eerder ook al is gevonden: het is lastig om duidelijke factoren te vinden die verantwoordelijk zijn voor verschillen in gevoeligheid. Zowel eerder onderzochte factoren (zoals sekse) als nog niet eerder door anderen onderzochte factoren (zoals stress van de moeder tijdens de zwangerschap) verklaarden weinig verschillen in gevoeligheid. Mogelijk zijn de oorzaken van verschillen in gevoeligheid zo complex dat ze moeilijk te achterhalen zijn in studies die slechts een of enkele factoren onderzoeken. Iets van ondersteuning hiervoor is te vinden in hoofdstuk 6. In dit hoofdstuk vonden we weliswaar noch een effect van stress bij de moeder rond de zwangerschap noch van genetische kenmerken, maar vonden we wel dat bij adolescenten die zowel veel stress rond de zwangerschap hebben meegemaakt als (veel) 'gevoelige' genen hebben, de relatie tussen stress en emotionele instabiliteit veel sterker is dan bij andere adolescenten. Deze groep van adolescenten was echter te klein om het effect statistisch in meer detail te kunnen onderzoeken, waardoor de bevinding met voorzichtigheid moet worden geïnterpreteerd.

Gevolgen van veranderingen in adaptieve capaciteit

In hoofdstukken 6 en 7 van dit proefschrift is gekeken hoe temperament en psychologische problemen voorspellend kunnen zijn voor het meemaken van stressvolle gebeurtenissen enkele jaren later. De studie beschreven in hoofdstuk 9 gaat verder op dit onderwerp in door te onderzoeken hoe *veranderingen* in temperament voorspellend kunnen zijn voor de ontwikkeling van psychische aandoeningen enkele jaren later. De resultaten laten zien dat veranderingen in temperament psychische aandoeningen voorspellen. Zo hebben adolescenten die een stijging laten zien in frustratie, een grotere kans om een psychische aandoening te ontwikkelen dan adolescenten die dalen in frustratie. De vraag is of ook veranderingen in HPA-as functioneren voorspellend zijn voor de ontwikkeling van psychische aandoeningen. Met de tot dan toe verzamelde TRAILS data hadden we (nog) niet de mogelijkheid deze vraag te beantwoorden.

Conclusie

In dit onderzoek staan effecten van stressvolle gebeurtenissen centraal. De belangrijkste bevinding is dan ook dat blootstelling aan stressvolle gebeurtenissen gerelateerd is aan non-normatieve veranderingen in temperament en HPA-as functioneren. Daarnaast laten de bevindingen zien, dat deze relatie wederkerig kan zijn; stress voorspelt niet alleen temperament, maar temperament is ook voorspellend voor het meemaken van stressvolle gebeurtenissen. Bovendien zijn veranderingen in temperament gerelateerd aan de ontwikkeling van nieuwe psychische aandoeningen. Er werd weinig bewijs gevonden voor verschillen in gevoeligheid voor de invloed van stress. Onze bevindingen zijn in tegenspraak met de traditionele assumptie van stabiliteit, vullen de studies naar normatieve veranderingen aan en, bovenal, benadrukken het belang van het bestuderen van veranderingen in temperament en HPA-as functioneren in de context van (stressvolle) gebeurtenissen.

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Odilia

Curriculum Vitae

Odilia Laceulle was born on November 5th, 1983 in Haarlem, the Netherlands. After having completed high school (VWO) in 2003, she did the bachelor Developmental Psychology (2004-2007) and the research master Development and Socialisation of Children and Adolescents at Utrecht University (2007-2009). Her master thesis was performed at the department of Developmental Psychology under the supervision of Prof. dr. M.A.G. van Aken and dr. A. Karreman, where she studied the influence of stressful life events on temperament development. For this thesis, data was used from TRAILS (TRacking Adolescents' Individual Lives Survey). This resulted in a PhD-project at the Interdisciplinairy Center Psychopathology and Emotion Regulation (ICPE), which she started while finishing her master thesis in 2009. Under supervision of Prof. dr. J. Ormel, Prof. dr. M.A.G. van Aken and dr. E. Nederhof, she investigated programming effects of adversity on adolescent adaptive capacity. An adapted version of Odilia's master thesis is included in the current thesis. During her PhD-project, Odilia visited respectively Prof. dr. V. Glover at the Imperial College, London (1 month) and Prof. dr. K.S.K. Kendler at the Virginia Commonwealth University (2 months). Odilia presented her research at several national and international conferences.

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